5th INTERNATIONAL SYMPOSIUM ON BACTERIAL CELLULOSE

22-23 September, 2022

Steigenberger Esplanade Jena hotel, Jena, Germany

5th ISBC

5th International Symposium on Bacterial Cellulose JENA 2022

WELCOME MESSAGE

Dear all,

On behalf of the Organizing Committee, we would like to warmly welcome you to the 5th International Symposium on Bacterial Cellulose (5ISBC)!

This event, which has been held biennially since 2013, mobilizes experts from the field, to discuss all aspects of research related to Bacterial Cellulose Biotechnology, including the biology and genetics of producing microorganisms, production biotechnologies and different areas of application, namely biomedicine, food and cosmetic industries, pharmaceutical applications, composites, pulp and paper. Previous editions took place in New Orleans (2013), Gdansk (2015), Fukuoka (2017) and Porto (2019).

The 5th ISBC will be held in Jena - Germany, at the Steigenberger Esplanade Jena hotel, between 22 and 23, September 2022.

As with previous events, this Symposium aims to attract international researchers and innovative start-ups to communicate and share the latest developments in this fast-moving and continually expanding field of Bacterial Cellulose.

Reasons to attend:
> Learn and share your knowledge with and from internationally renowned researchers
> Understand the current state of research and the challenges to future discovery.
> Meet and socialize with fellow scientists from around the world
> Meet successful start-up companies with innovative BC based products.
> Take some time to explore the historic city of Jena and its beautiful surroundings!

We believe the next few years will witness the translation of BNC from Academia to the Bioeconomy. Be a part of it and contribute to this exciting challenge!

Dana Kralisch and Dieter Klemm

Chairs for the 5th International Symposium on Bacterial Cellulose

5ISBC 2022, Jena, Germany

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SPONSORS

DETAILED PROGRAM

21 September

19h	Welcome gathering, city tour

22 September Day 1

8:00h	Registration
8:45h	Opening ceremony

SESSION 1	**CHARACTERIZATION, MODIFICATION AND TOXICOLOGY** chairperson: Dana Kralisch
9h00	**Keynote lecture: Amphiphilic Janus-type bacterial cellulose nanofibrils prepared by aqueous counter collision** Tetsuo Kondo, Kyushu University, Japan
9h30	**Visualizing Bacterial Cellulose Nanostructure through High Resolution Microscopy** Jose Moran-Mirabal, McMaster University, Canada
9h45	**Prediction of mechanical property for bacterial cellulose hydrogel using quantitative analysis of fiber network structures** Go Takayama, Kyushu University, Japan
10h00	**Bacterial Nanocellulose: Functional relationships between viscoelastic properties and manufacturing processes using a novel measurement and evaluation strategy** Holger Rothe, Institute for Bioprocessing and Analytical Measurement Techniques e.V., Germany
10h15	**Controlling BC morphology through a simple biosynthesis step** Anna Laromaine, Institut Ciencia de Materials de Barcelona, Spain
10h30	**Overcoming the regulatory hurdles on nanocelluloses – is bacterial cellulose absorbed at the intestine?** Miguel Gama, University of Minho, Portugal
10h45	*Coffee break*

SESSION 2	**COMPOSITES AND APPLICATIONS, PART 1** chairperson: Falk Liebner
11h15	**Keynote lecture: Structures, Properties, and Applications of Nanofibrillated Bacterial Cellulose (NFBC) (online)** Kenji Tajima, Hokkaido University, Japan
11h45	**Formulation of biohybrid materials with different functionalities through biotechnological approaches** Maria Auxiliadora Jimenez, National Spanish Research Council-CSIC, Spain
12h00	**Bacterial cellulose nanocrystals and polyphenolic extract from grape pomace for Pickering emulsions** Julen Diaz Ramirez, University of the Basque Country, Spain

12h15	**Growing Whole Bacterial Cellulose Garments with Membrane Technologies und Industrial Robotics** Emanuel Gollob/Miriam Eichinger, University of Art and Design Linz, Austria
12h30	**Constructing ultrathin, strong, and highly flexible MXene/bacterial cellulose composite films by a scalable biosynthesis method (online)** Quanchao Zhang, East China Jiaotong University, China
12h45	**Bacterial cellulose as an alternative textile for apparel (online)** Jane Wood, Manchester Metropolitan University, England
13h00	*Lunch*

SESSION 3	**BIOMEDICAL APPLICATIONS, PART 1 – IMPLANTS & TISSUE ENGINEERING** chairperson: Inder Saxena
14h30	**Recent clinical experiences with biosynthetic cellulose in burn treatment – A review** Dana Kralisch, JeNaCell, Germany
14h45	**How to Optimize Coronary Artery Bypass Graft Prosthesises based on Bacterial Nanocellulose** Jorn Hulsmann, Medical Faculty, Magdeburg, Germany
15h00	**In vivo evaluation of bacterial cellulose for abdominal hernia repair and MRI monitoring** Anna Roig, Institut Ciencia de Materials de Barcelona, Spain
15h15	**Conducting Bacterial Nanocellulose-Polypyrrole (BC-Ppy) scaffolds for cardiac tissue engineering** Sumithra Srinivasan, Institut Ciencia de Materials de Barcelona, Spain
15h30	**Bacterial cellulose membranes loaded with bioactive compounds formulated in neoteric solvents: a joint strategy to improve their biological efficacy** Armando Silvestre, University of Aveiro, Portugal
15h45	**Hydrating patches from bacterial nanocellulose – a platform approach for the treatment of nail diseases** Tom Bellmann, Friedrich-Alexander-Univ. Erlangen-Nürnberg, Germany
16h00	**Potential of bacterial nanocellulose in reconstructive surgery of body integumentary system - preliminary studies in animals (online)** Agata Błażynska, Gdansk Medical University, Poland
16h15	*Coffee break*

SESSION 4	**MOLECULAR TOOLS AND BIOSYNTHESIS** chairperson: Stanislaw Bielecki
16h45	**Keynote lecture: Mutant analysis for understanding cellulose biosynthesis in *Gluconacetobacter xylinus*** Inder Saxena, The University of Texas at Austin, USA
17h15	**Size modulation of bacterial cellulose spheroids in *Komagataeibacter xylinus*** Satomi Tagawa, Shinshu University, Japan
17h30	**Draft genome analysis of new strain of *Komagataeibacter xylinus* isolated from the production environment of fruit vinegar** Masahiro Mizuno, Shinshu University, Japan
17h45	**A synthetic biology approach to growing a photograph (online)**

	Tom Ellis, Imperial College London, England
18h00	**Control of bacterial cellulose biosynthesis guided by systems-level insights (online)** Małgorzata Ryngajłło, Lodz University of Technology, Poland
18h15	END OF DAY 1
19h30	*Symposium dinner*

23 September Day 2

SESSION 5	**BIOMEDICAL APPLICATION, PART 2 – WOUND DRESSINGS & DRUG DELIVERY** chairperson: Anna Roig
9h00	**Keynote lecture: Bacterial nanocellulose based materials for dermal applications** Carmen Freire, CICECO/University of Aveiro, Portugal
9h30	**Drug Delivery Strategies using Bacterial Nanocellulose: What is next?** Dagmar Fischer, Friedrich-Alexander-Unviversität Erlangen-Nürnberg, Germany
9h45	**Tailoring Bacterial Cellulose dressings to the requirements of chronic wound care** Paul Zahel, JeNaCell - An Evonik Company, Germany
10h00	**Multifunctionalization of bacterial nanocellulose for improved wound dressing properties** Falk Liebner, University of Natural Resources and Life Sciences, Austria
10h15	**Elastic Tubular Bacterial Nanocellulose Composites for Small-caliber Vascular Grafts (online)** Feng Hong, Donghua University, China
10h30	**Novel Functional Bacterial Cellulose composites used for Tissue Regenaration and Repair (online)** Yudong Zheng, University of Science and Technology Beijing, China
10h45	*Coffee break*

SESSION 6	**FERMENTATION** chairperson: Tetsuo Kondo
11h15	**Keynote lecture: Bacterial Cellulose production: valorization of wastewater and Life Cycle Assessment** Fernando Dourado, Satisfibre/University of Minho, Portugal
11h45	**High-yield Production of Nanofibrillated Bacterial Cellulose (NFBC) in Aerated and Stirred Bioreactors** Ryo Takahama, Hokkaido University, Japan
12h00	**Bacterial nanocellulose production from carbon dioxide** Silvia Marqués, Estación Experimental del Zaidin, Spain
12h15	**Bacterial nanocellulose-based composites as eco-innovative materials with potential industrial and medical significance (online)** Monika Kaczmarek, Lodz University of Technology, Poland
12h30	*Lunch and group photo*

SESSION 7	**COMPOSITES AND APPLICATIONS, PART 2** chairperson: Dieter Klemm
14h00	**Reinforced natural hydrogels of collagen and bacterial nanocellulose for 3D culture of mesenchymal stromal cells** Nanthilde Malandain, Institut Ciencia de Materials de Barcelona, Spain
14h15	**Bacterial cellulose is 'alive': Practitioners' experiences developing bacterial cellulose as a versatile emerging biomaterial**

	Luis Quijano, Queensland University of Technology, Australia
14h30	**Bacterial Cellulose Nanofibrils as a Novel Vaccine Adjuvant** Ozge Suer, Izmir University of Economics, Turkey
14h45	**Development of layered BNC composites for Food Packaging** Francisco Garrett, University of Minho, Portugal
15h00	**Use of functionalized bacterial cellulose/fibroin as conformable substrate in organic field-effect transistors (OFETs) (online)** Arthur Barreto, Pontifícia Univ. Católica do Rio de Janeiro, Brazil
15h15	**Argon Plasma-Modified Bacterial Cellulose Filters for Protection Against Respiratory Pathogens (online)** Anna Zwica, West Pomeranian University of Technology, Poland
15h30	*Coffee break*

SESSION 8	**INDUSTRIAL LANDSCAPE** chairperson: Miguel Gama
16h00	**Keynote lecture: Closing the gap to profitable commercialization of bacterial cellulose-based technologies: established large-scale bacterial cellulose production and product form optimization** Yves Boland, CPKelco, USA
16h30	**KKF – polymers for life: Biotechnologically designed bacterial cellulose materials with application-oriented shape, surface and network structures** Vanessa Raddatz, KKF polymers, Germany
16h45	**Uncover the untapped potentials of fermented (bacterial) cellulose: application for coatings and cosmetics** Deby Fapyane, Cellugy ApS, Denmark
17h00	**A new generation Bacterial Cellulose based Hydrocolloid for Food Industry** Srinivas Karuturi, BIOWEG UG, Germany
17h15	**The Hylomorph journey from bench to bedside (online)** Aldo Ferrari, Hylomorph AG, Switzerland
17h30	**From lab to market – How to build up a successful business?** Dana Kralisch, JeNaCell - An Evonik Company, Germany
17h50	***END OF SYMPOSIUM / NEXT SYMPOSIUM***

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ORAL COMMUNICATIONS: 22 September Day 1

SESSION 1 - CHARACTERIZATION, MODIFICATION AND TOXICOLOGY (chairperson: Dana Kralisch)

KEYNOTE LECTURE: Amphiphilic Janus-type bacterial cellulose nanofibrils prepared by aqueous counter collision

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Abstract

The authors have extensively developed a nanopulverization process to yield a single nanofibril, termed aqueous counter collision (=ACC) method, for various bio-based resources including wood, Bacterial cellulose (BC) pellicle, bamboo, collagen and so on, solely using the impingement of two high-speed jets of aqueous suspension of raw materials expelled through a pair of opposing nozzles[1-3]. Among the resources, BC pellicle has a disadvantage of its high cost for the production, although it exhibits excellent mechanical properties and biocompatibility. Therefore, the application would be limited for the well suited high value-added products like medical and pharmaceutical materials, as well as food and cosmetics industry and traded worldwide[4].

To expand the application areas in general, two different production designs should be considered to counterbalance usage and cost, namely, “high-price and low-loading” or “low-price and high-loading”. Thus, with regards to application of BC pellicle and its single nanofibril (=ACC-BNC), the present direction described above would be reasonable, and simultaneously it is no doubt of importance to improve biotechnological cultivation techniques. In addition of the above normal concept, however, further expansion of the application requires a concept of“high-price, but low-loading for unique functions induced by BC”.

Recently, the detailed process of the ACC nanopulverization method has been revealed. Namely, the kinetic energy of the impinging due to ACC, which can be converted into elastic–plastic waves, remains higher than the van der Waals forces and weak hydrogen bonds typically at an ejection pressure of 200 MPa[1,2]. The propagation of shock waves resulting from ACC occurs through elastic crystalline domains to cleave the hydrophobic van der Waals planes normally hidden inside native crystalline cellulose fibers with these hydrophobic planes eventually being exposed on the surface.

The resulting ACC–CNFs have a Janus-type amphiphilic surface composed of both hydrophobic and hydrophilic planes in an aqueous system[5]. BC pellicle, which is highly crystalline, is an idealistic raw material for ACC that exhibited such amphiphilic properties[5,6]. In highly crystalline BC, the ACC shock waves are simply propagated through the elastic crystalline domains from the initial stage, resulting in the direct cleavage of certain interfaces.

More recently, it was found that oil-in-water Pickering emulsions with long-term stability are readily prepared by ultrasonic mixing of aqueous ACC–BNC dispersions with nonpolar solvents[7]. The emulsification abilities of ACC–BNC for stable Pickering emulsions have been proven to be significantly greater than other CNFs prepared in different manners. As a further development, ACC–BNC was found to preferentially adsorb onto hydrophobic isotactic polypropylene microparticles and other plastic particles, which demonstrates their characteristic amphiphilicity[8]. The coted plastic particles with extremely small quantity of CNF could be converted plastic products with super impact-tolerant plastics.

This presentation will attempt to summarize the above story, and hereafter future perspectives using ACC-BNC coated plastic particles.

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Visualizing Bacterial Cellulose Nanostructure through High Resolution Microscopy

Mouhanad Babi,[1] Ayodele Fatona, [1] Anthony Palermo, [1] Alyssa Williams,[2] Xiang Li, [1] Christine Cerson, [1] Victoria Jarvis,[3] Nabil Bassim,[4,5,6] Emily Cranston,[7,8] Tiffany Abitbol,[9] Jose Moran-Mirabal[1,2,6,10]

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Abstract

Cellulose is the most abundant structural polymer in nature and, due to its unique mechanical and chemical properties, has been used as a source of building materials and paper products. Through research, we are learning that crystalline nanocellulose (*i.e.,* bacterial cellulose – BC, cellulose nanocrystals – CNCs, and cellulose nanofibrils, CNFs) not only exhibits the expected enhanced mechanical properties, but also displays unique optical, electric, and magnetic properties that are not found in macroscale cellulosic materials. These unique properties, have made nanotechnologists call cellulosic nanomaterials ''nature's carbon nanotubes.'' [1]

Nanocelluloses are attractive materials for the production of biodegradable and renewable products. The use of nanocelluloses for such practical applications often requires visualizing their distribution within complex matrices. This can be achieved with fluorescence microscopy techniques if the nanocelluloses are fluorescent, as the sensitivity and specificity of these methods permit visualizing nanoparticles and nanofibers within complex systems. Yet, advanced imaging methods like multiphoton or super-resolution microscopy are rarely applied to cellulose. This is partly due to a lack of efficient and cost-effective methods to fluorescently tag nanocelluloses without altering their unique properties. We have developed modular surface chemistry, based on triazinyl linkers, that allows us to tune the reactivity of cellulosic materials, leading to efficient ways of fluorescently tagging nanocelluloses.[2]

In this presentation, I will describe our work on labeling nanocelluloses for use in high resolution fluorescence microscopy studies. I will present a versatile and efficient two-step approach based on triazine and azide–alkyne click-chemistry to label nanocelluloses with a variety of dyes.[3] This method was used to label BC fibrils, and plant-derived CNFs and CNCs to high degrees of labeling using minimal amounts of dye while preserving their native morphology and crystalline structure. The ability to tune the labeling density with this method allowed us to prepare optimized samples that were used to visualize nanostructural features of cellulose through super-resolution microscopy. Using this approach, we directly visualized and measured alternating crystalline and disordered regions within individual fluorescently labelled BC fibrils.[4] To investigate the structural origins of the disordered regions along BC fibrils, we used correlative super-resolution light/electron microscopy and observed that the disordered regions seen in super-resolution correlate with the ribbon twisting observed in electron microscopy.[5] Through efficient labeling and high-resolution microscopies, unraveling the hierarchical assembly of bacterial cellulose and the ultrastructural basis of its disordered regions provides insight into its biosynthesis and susceptibility to hydrolysis.

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Prediction of mechanical property for bacterial cellulose hydrogel using quantitative analysis of fiber network structures

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Abstract

Bacterial cellulose (BC) pellicle having engagements of the secreted nanofibers exhibits excellent mechanical properties and biocompatibility. The advantages lead BC pellicle to a promising soft matter for various applications, and thus many studies have been conducted to engineer BC based materials[1]. For further advancements, there is a crucial problem in quality control of BC pellicle, since BC fiber structures produced are strongly dependent on the microbial culture conditions such as the bacterial strains and culture environments[2]. Such flexibility of properties in BC pellicles complicates the comprehensive understanding and facile regulation of BC based materials, although three-dimensional network structure of cellulose ribbon is believed as an important structural factor determining physical property of BC pellicle. As a most feasible approach to solve the above, quantitative analysis of network structure can be listed for prediction of mechanical properties related to the conditions of BC production. Here, we attempt to quantitively evaluate the 3D network structure of BC pellicle using fluorescent microscopy–based image analysis to verify the relationship between network structure and physical property of BC pellicle.

Experimental

Seven strains of genus *Komagataeibacter* bacterium were cultured to prepare BC pellicles with varied morphology. The prepared pellicles were stained with Fluorescent Brightener 28 (Sigma Aldrich) for obtaining confocal laser scanning microscopic images as 3D structural data of fiber network. Then, fiber structure were segmented using 3D fiber segmentation soft SOAX[3] before fiber cross-link density of the pellicles were calculated. Separately, mechanical property of pellicles was evaluated using dynamic mechanical analysis in aqueous environments.

Results and discussion

Both calculated cross-link density and measured storage modulus *E*' for pellicles varied within the right Figure depending on the bacterial strains. Figure indicated that the robust regression analysis revealed its linear relationship. This result is in a good agreement with the hypothesis that fiber cross-link density determines the mechanical properties of BC pellicle. This insight further leads to the possibility of simple and robust method of predicting mechanical properties of BC pellicles without special mechanical testing apparatus and would accelerate the research and application of BC based materials.

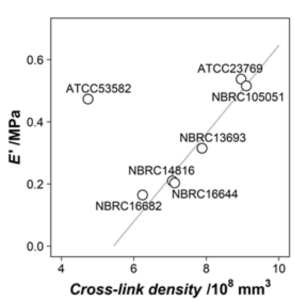

Figure. Correlation between cross-link density and storage modulus *E*' for various strains of *Komagataeibacter*. Linear regression line based on robust estimation were visualised.

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Bacterial Nanocellulose: Functional relationships between viscoelastic properties and manufacturing processes using a novel measurement and evaluation strategy

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Abstract

Bacterial nanocellulose is a highly interesting biomaterial with some unique properties such as bioinertness, high porosity and swelling capacity, and high elasticity under tensile stress.

The excellent elastic properties under tensile stress are in contrast to the largely viscous behaviour under compressive load, which is not suitable for a range of applications (e.g. wound dressings) [1].

In the past, many studies have therefore been carried out with the aim of stiffening BNC under compressive load. The latter was achieved on the one hand by a targeted variation of cultivation methods, where dynamic methods such as the mobile matrix reservoir (MMR) technique have proven to be promising, and on the other hand by post-modification of BNC nonwovens by crosslinking, functionalization with additives, dehydration or drying [2-12].

Nevertheless, no measurement and evaluation strategy existed so far to clearly assign the effect of the undertaken modifications on the viscoelastic behaviour. The latter applies to soft-matter biomaterials in general and to BNC in particular.

Here we present a measurement and evaluation strategy that allows to derive the effects of individual manufacturing or post-modification strategies on the viscoelastic behaviour based on easy to perform relaxation measurements after initial pressure application.

Applying our method, relaxation data are fitted using an evolutionary algorithm based on a viscoelastic model implementing a continuous distribution of relaxation times. Using this method, we were able to make explicit assignments of manufacturing parameters and post-modifications to the relaxation spectrum.

We show that the cultivation conditions for BNC primarily affect the height of dispersion and the frequency of the relaxation centre, and that these effects can be further enhanced by post-modifications. However, we also identify parameters, such as the width of the relaxation region, on which the type of cultivation obviously shows no influence but which can be influenced exclusively by post-modifications [13].

Our methodology enables for the first time a clear identification of those parameters which represent a significant factor of influence to the viscoelastic material behaviour, which should enable a more targeted and application-relevant development of BNC composites in the future.

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Controlling BC morphology through a simple biosynthesis step

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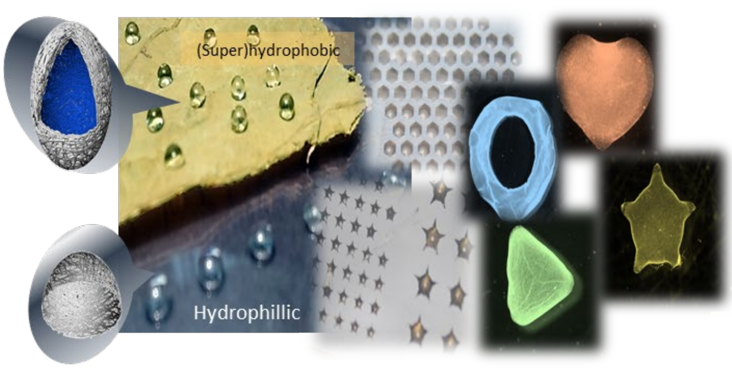
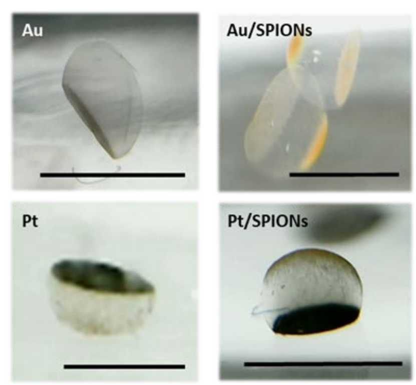
Abstract

Cellulose and bacterial nanocellulose (BNC) are promising building blocks for high-performance biomaterials due to their remarkable properties such as high water holding capacity, porosity, mechanically solid, elastic, and biocompatibility.
Those extraordinary properties placed BNC at the intersection of different applications, especially if we can control the intrinsic properties of the cellulose fibrils mesh, such as their arrangement and their interactions with proteins and solvents, or the addition of additives to endow novel properties to the material, among others. Understanding the primary material and the novel composites endorses its uses in fields ranging from batteries to skin grafts or even heart replacement pouches.
This work presents how a single BNC´s biosynthesis step in static allows us to obtain spheres and aligned cellulose reproducibly and straightforwardly, avoiding complex equipment or chemical additives.
Our methodologies, based on one-step BNC biosynthesis, and the characterization of BNC spheres, BNC decorated with nanoparticles, Janus BNC spheres, and aligned BNC will be presented.





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Overcoming the regulatory hurdles on nanocelluloses – is bacterial cellulose absorbed at the intestine?

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Abstract

The potential of nanomaterials in food technology is nowadays well-established. However, their commercial use requires a careful risk assessment, in particular concerning the fate of nanomaterials in the human body. Bacterial NanoCellulose (BNC), a nanofibrillar polysaccharide, has been used as a food product for many years in Asia. However, given its nano-character, several toxicological studies must be performed, according to the European Food Safety Agency's Guidance. Those should especially answer the question on whether nanoparticulate cellulose is absorbed in the Gastrointestinal Tract. This raises the need to develop a screening technique capable of detecting isolated nanosized particles in biological tissues.

Here, the potential of a Cellulose Binding Module fused to a Green Fluorescent Protein (GFP-CBM) to detect single bacterial cellulose nanocrystals (BCNC) obtained by acid hydrolysis was assessed. Adsorption studies were performed to characterize the interaction of GFP-CBM with BNC and BCNC. Correlative Electron Light Microscopy was used to demonstrate that isolated BCNC may be detected by fluorescence microscopy. The uptake of BCNC by macrophages was also assessed. Finally, an exploratory 21-day repeated dose study was performed where Wistar rats were daily fed with BNC. The presence of BNC or BCNC throughout the GIT was observed only in the intestinal lumen, suggesting that cellulose particles were not absorbed. While a more comprehensive toxicological study is necessary, these results strengthen the idea that BNC can be considered a safe food additive [1].

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SESSION 2 - COMPOSITES AND APPLICATIONS, PART 1 (chairperson: Stanislaw Bielecki)

KEYNOTE LECTURE: Structures, Properties, and Applications of Nanofibrillated Bacterial Cellulose (NFBC)

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Abstract

Nanofibrillated cellulose (NFC) has been receiving attention as a new material. NFC is generally produced from pulp via a top-down process, and various physical and/or chemical production processes have been reported. In contrast, we have developed a different NFC (NFBC) production system via a bottom-up process using a cellulose-producing bacterium [*Gluconacetobacter intermedius* (*G. intermedius*) NEDO-01] [1]. In this process, a cellulose-producing bacterium was cultured with aerobic agitation in a medium supplemented with carboxymethylcellulose (CMC) as a dispersing agent of microfibrils. The NFBC prepared with CMC (CM-NFBC) showed high dispersibility in water but rapidly aggregated in organic solvents. To overcome this problem and expand its applicability, we have also succeeded in preparing an amphiphilic NFBC (HP-NFBC) via a one-step process by using amphiphilic hydroxypropylcellulose (HPC) instead of CMC, which improved the dispersibility of NFBC in organic solvents. [2] Until now, detailed structural information such as fiber length, fiber length distribution, and viscoelastic properties of those NFBCs have not been obtained.
In this presentation, I will introduce structural information and viscoelastic properties of NFBCs and applications that take advantage of the NFBC's features.

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Formulation of biohybrid materials with different functionalities through biotechnological approaches

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Abstract

One of the most promising advantages of bacterial biopolymers is the fact that their physical and chemical properties can be custom-designed for each specific application through the use of microbial biotechnology tools 1. On the one hand, bacterial cellulose (BC), an extracellular biopolymer produced by bacteria of the genus *Komagataeibacter spp*., has been widely used in view of its excellent mechanical performance with a high capacity of retaining water in its nanofibrillar structure. On the other hand, polyhydroxyalkanoates (PHA), an intracellular biopolymer synthetized by bacteria of many genus, highlight by their hydrophobic character as well as high flexibility, which triggers this material has excellent barrier properties. The fact that these two biopolymers have quite opposite properties has made researchers have the intention of using them in a complementary manner, but it has triggered the main limitations of this process as well, related to their incompatibility.

This work aims to design customized materials with compartments of different functionalities involving the complementary use of BC and PHA and carrying active cargos, such as enzymes and/or microorganisms. This novel approach would allow the interaction of these two biopolymers as well as with the cargos through different bonding types, including physical and/or chemical interactions. These biohybrid materials can suppose a step towards the fabrication of engineered living materials with customized properties that could be applied in a very diverse field of applications.

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Bacterial cellulose nanocrystals and polyphenolic extract from grape pomace for Pickering emulsions

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Abstract

Bacterial cellulose nanocrystals (BCNCs) are known for their great applicability in various fields, including their use in the stabilisation of Pickering emulsions due to their sustainability, green condition and amphiphilic nature [1,2]. In Pickering emulsions, surfactants are replaced by solid particles, reducing the risk of coalescence and with an adjustable permeability [3]. As BCNCs are easier to be hydrated and well-dispersed in aqueous medium than other types of cellulose nanocrystals, they are capable of emulsifying a wide range of oil varieties [4].

Moreover, in addition to stabilising the emulsion on their own, BCNCs can create synergistic complexes with other substances to improve stability and provide additional properties [5]. Thus, the interaction between cellulose and polyphenols can be exploited to improve the oxidative stability of lipids in oil in water emulsions[6].

In this work, BCNCs were successfully obtained from bacterial cellulose (BC) biosynthesized from grape pomace culture media. Characterization of BCNCs obtained both by acidic hydrolysis (AH) and enzymatic hydrolysis (EH) proved their high crystallinity, nanometer scale and good thermal stability. Furthermore, zeta potential measurements showed that the interaction with grape pomace polyphenolic extract (GPPE) increased the negative charge of the BCNCs. In this way, the BCNC-GPPE system confirmed adequate colloidal properties to be used as stabilizers of Pickering emulsions. In addition, the free radical scavenging activity of different BCNC-GPPE combinations was determined against time. As a result, all BCNC-GPPE samples exhibited antioxidant capacity for more than 40 days under different light and temperature conditions.

BCNCs and BCNC-GPPE complexes were used to form hexadecane in water Pickering emulsions. Then, the emulsion rate and the particle size in the emulsified phase were studied after one and seven days, respectively. Both BCNCs obtained from AH and EH showed emulsifying capacity in a dose-dependent manner. The addition of GPPE had a positive effect on emulsion stabilization, especially noticeable in the case of BCNCs from EH. Finally, olive oil Pickering emulsions stabilized by BCNCs and BCNC-GPPE were subjected to Schaal Oven Test in order to measure the increase of conjugated dienes. The results showed that when GPPE was present, the oxidative stability was considerably higher. This work contributes to an advanced revaluation of an agricultural waste such as grape pomace. The BCNC-GPPE complex could be a Pickering emulsion stabilizer of special interest for the food and cosmetic industry.

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Growing Whole Bacterial Cellulose Garments with Membrane Technologies und Industrial Robotics

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Abstract

The world of fashion is undergoing the most significant phase of upheaval since industrialization. The current fashion industry practice results in a highly negative societal and environmental impact. In reaction to these adverse effects, a new wave of designers and artists are exploring the potential of biomaterials such as algae and bacteria as an alternative to oil- and cotton-based textiles.

The British fashion designer Suzanne Lee pioneered the introduction of Kombucha Leather in the textile sector in 2004 [1]. Her bio fabrication research led us in the direction of the already well-known model organism *Komagataeibacter Xylinus*, which is a bacterial cellulose producer and could potentially offer an alternative to cotton-based garments. Growing cotton plants industrially is very water-intensive and demands lots of pesticides. The industrial standard of processing cotton into fabric involves environmentally harmful bleaching, colouring, and finishing chemicals. Besides that, fabricating two-dimensional pattern pieces before sewing them into three-dimensional garments generates additional fabric waste and demands joining methods like seams, predetermined breaking points in a clothing's life cycle.

In reaction to these challenges, we present a method to grow whole bacterial cellulose (BC) garments in textile membranes three-dimensionally directly. Medical research showed it is possible to grow medical implants with seamlessly connected bacteria cellulose in silicone moulds [2]. When targeting human proportions, silicone is not a feasible membrane material due to its elasticity. In contrast, textile membranes are available with lower elasticity and higher oxygen permeability. Therefore, this paper explicitly investigates the use of membrane textiles as growing moulds. The membrane containers function as a bioreactor in which the bacteria metabolize sugar and oxygen into cellulose and CO2.

Such a garment-shaped membrane container allows growing spatial bacterial cellulose pellicles with an even wall thickness. Like highly functional sports apparel, clothing has to fulfil a diverse range of functions. Those functions are usually non-evenly spread along the body. Finding ways to locally tweak nutrition and temperature in the growing process of the BC pellicle could allow interweaving fabric attributes gradually. Knowledge about programming material features through physical interaction is one of the strengths of parametric robotics. This leads to the second research goal of this paper, the objective to explore the potential of utilizing industrial robotics for interacting with bacterial celluloses growth through local real-time increase of oxygen flow by micro punctuation of the textile bioreactors.

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Constructing ultrathin, strong, and highly flexible MXene/bacterial cellulose composite films by a scalable biosynthesis method

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Abstract

Significant efforts have been made to develop high-performance electromagnetic shielding materials to mitigate the electromagnetic radiation generated by telecommunication and electronic devices [1]. However, the fabrication of ultrathin films that are electrically conductive and mechanically strong for electromagnetic interference (EMI) shielding applications remains a huge challenge. In this work, ultrathin, strong, and highly flexible $Ti_3C_2T_x$ MXene/bacterial cellulose (BC) composite films were fabricated by a scalable *in situ* biosynthesis method. The $Ti_3C_2T_x$/BC composite films exhibited an integrated structure with uniformly distributed $Ti_3C_2T_x$ nanosheets in the BC network and mechanical entanglement between the 2D $Ti_3C_2T_x$ nanosheets and the one-dimensional BC nanofibers, which endowed the $Ti_3C_2T_x$/BC composite films with excellent conductivity (electrical conductivity was 15625 S m^{-1} at 76.9 wt% $Ti_3C_2T_x$), high mechanical properties (tensile strength was 297.5 MPa at 25.7 wt% $Ti_3C_2T_x$) and flexibility. Importantly, a 4 µm thick $Ti_3C_2T_x$/BC composite film with 76.9 wt% $Ti_3C_2T_x$ content demonstrated a specific EMI shielding efficiency of 29141 dB cm^2 g^{-1}, which surpasses those of most previously reported MXene-based polymer composites with similar MXene contents and carbon-based polymer composites. Furthermore, absorption and reflection were found to be the main contributors to the shielding performance because of the high conductivity and EM wave absorption ability of the $Ti_3C_2T_x$ nanosheets. It is worth noting that $Ti_3C_2T_x$/BC composite promoted cell adhesion, proliferation, and differentiation, which exhibited good biocompatibility. Our findings suggest that the facile, environmentally friendly, and scalable fabrication method is a promising strategy for producing ultrathin, strong, and highly flexible EMI shielding materials such as the free standing $Ti_3C_2T_x$/BC composite films for efficient EMI shielding to address EMI problems of a fast-developing modern society.

Keywords: MXene, Bacterial cellulose, Biosynthesis, Electromagnetic interference shielding

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Bacterial Cellulose as an Alternative Textile for Apparel

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Abstract

By 2030 global apparel consumption is estimated to be 102 million tons, with the United Nations estimating the equivalent of approximately three planets worth of resources required by 2050 to sustain such demand [1]. The impact of textiles on the environment is wide reaching; every stage of the manufacturing process is resource hungry, from chemicals used in fibre production, processing techniques in fabric creation and manufacturing practices in apparel construction. Additionally, environmental pollution is continued during the lifetime of the garment due to washing and wear (detergents and microfibres) and estimates of over 300 thousand tonnes of clothing being discarded every year with approximately 20% of this sent to landfill [2]. The textile industry is seeking alternative sources of raw material and new production processes to address environmental concerns.

Bacterial cellulose (BC) is one such material for consideration. Its production in pure form without contaminants eliminates the need for the heavy processing required to remove such impurities in traditional textiles [3]. Its ease of production and ability to be moulded into shape has led practitioners to suggest BC could be used as a 'vegetable leather' in apparel; however there has been very little physical analysis of BC as a textile in this context [4, 5].

This study evaluated the performance of laboratory grown BC sheets when tested to international standard test methods commonly used for apparel fabrics; tensile strength and elongation (BS EN ISO 13934), abrasion (BS EN ISO 12947-1) and breathability (BS EN ISO 7209). Comparison fabrics (animal leather, animal suede, wool, polyamide non-woven, plant cellulosic non-woven) were chosen according to similarities in mass per unit area, fibre composition and fabric construction.

The BC sheets behaved in a similar manner to animal leather in tensile and elongation testing. The force required to break BC was lower than the animal leather and suede but was higher than the other non-woven structures. BC was the least breathable of all the samples tested but displayed the greatest ability to retain water. However, BC outperformed all samples in the abrasion test, showing the highest resistance to breakdown and exceeding industry standards.

Therefore, whilst the tensile and breathability results illustrate the limitations of BC as an apparel textile, the outstanding performance of BC to abrasion testing suggests it could have some applications in protective and performance clothing.

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SESSION 3 - BIOMEDICAL APPLICATIONS, PART 1 – IMPLANTS & TISSUE ENGINEERING (chairperson: Inder Saxena)

Recent clinical experiences with biosynthetic cellulose in burn treatment – A review

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Abstract

The benefits of biosynthetic cellulose in wound healing are known for quite some time. Pioneers in the field like Fontana[1] and colleagues as well as Czaja and colleagues[2] reported early about immediate pain relief, good and close adhesion to the wound bed, a barrier function against infection, easiness of wound inspection, healing support, improved exudate management and reduced time of treatment, as well as reduced costs. However, never before such a number of internationally renowned burn specialist and plastic surgeons reported about their own experiences and clinical observations made with a biosynthetic cellulose wound dressing, namely epicite[hydro] (producer: JeNaCell GmbH). An overview about those clinical observation studies, outcome in comparison to alternative materials typically used in burn wound treatment and general learnings based on the observation of larger patient groups will be given.[3-7] In addition, new scientific findings with respect to factors influencing wound healing and dermal tissue remodeling will be highlighted.[8]

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How to Optimize Coronary Artery Bypass Graft Prosthesises based on Bacterial Nanocellulose

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Abstract

Coronary artery bypass grafting (CABG) installs a complex and artificial biophysical niche to the treated heart. Respectively, clinically oriented biomaterial research efforts on CABG prosthesises are largely focused on designing bridges to this niche while minimizing a loss in biocompatibility. Another option is tailoring the biophysical properties of a graft towards matching them to a biological compatibility specific for this artificial niche.

Today, impacting biological niches by biophysical properties as well as functional micro- and nano-architectures are well studied, so a broad knowledge of single effectors is available. But to transfer this knowledge to feasible clinical applications, simple integrative processing technology that enables to adopt, combine, and adapt functional attributes is missing. Interestingly, growing Bacterial Nanocellulose (BNC) biofilms provide this integrative character elegantly by its natural freedom in form and intrinsic architecture.

By MMR-Tech coating [1], both macroscopic and intrinsic architecture of bacterial biofilms result from a progression of interdisciplinary sub-processes, each reflected by a specific stand-alone subsystem of variables and parameters. In the course of the mentioned progression, these variables get interconnected and finally define the hydrogels bifunctional attributes. At the same time, any isolated optimization of single functional aspects of the BNC hydrogel scaffold are hampered.

In this talk, insights of an approach to still get into modular control via complementing standard empirical biomedical evaluations by bioprocess-control methodology are presented. To identify and optimize critical variables inside of the natural integral of the growing biofilm, the MMR-Tech progression is approximated by a classical control path. On this pictural level, single aspects can be optimized separately.

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In vivo evaluation of bacterial cellulose for abdominal hernia repair and MRI monitoring

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Abstract

A tissue reinforcement is often required in the surgical management of abdominal hernias. Hernia prevalence is high; affecting 5% of the general population at least once during their lifetime[1]. Strengthening permanently the abdominal wall with a thin surgical mesh –known as hernioplasty– has become the mainstay for hernia repair. Hernioplasty has prevailed over approaches relying on sutures owing to the reduced recurrences achieved with this method.[2] The majority of the implantable meshes are constructed from synthetic non-resorbable polymers and will remain indefinitely inside the body. Nevertheless, hernia repair surgery is not exempt from post-operative complications. Adverse events can be related to infections or hernia reappearances, but also arise from intraperitoneal adhesions which are formed by scar-like tissue sticking together the implanted mesh and the adjacent organs. These adhesions are a major cause of chronic pain after hernia repair and a common trigger of secondary surgeries.

We will report on the evaluation of bacterial cellulose (BC) as a soft tissue reinforcement material. A double-layer BC laminate proved sufficient to meet the standards of mechanical resistance for abdominal hernia reinforcement meshes while BC-polypropylene composites incorporating a commercial mesh could also be prepared (see Figures). The *in vivo* study of implanted BC patches in a rabbit model exhibited excellent anti-adherent characteristics.[3] Besides, we will present simple approaches to pattern inorganic nanoparticles on the surface of a BC film and demonstrate their monitoring by magnetic resonance imaging (MRI).[4] In summary, we advocate BC as a potentially useful biomaterial for hernioplasty with excellent mechanical and anti-adherent characteristics which can incorporate medical imaging and antioxidant capabilities.

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Conducting Bacterial Nanocellulose-Polypyrrole (BC-Ppy) scaffolds for cardiac tissue engineering

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Abstract

The human body consists of electrically conducting organs such as the heart and nervous system. The heart is composed of muscle cells called cardiomyocytes. When the blood supply to a region is blocked, it leads to an irreversible condition called as myocardial infarction(MI). In the case of MI, the infarcted region becomes non-conductive, resulting in a disturbance in electrical propagation leading to cardiac arrhythmia (CA). Biocompatible scaffolds on the affected region as a cardiac patch can facilitate inter-cellular communication between the healthy regions and resynchronise CA [1]. Therefore, electrically conducting polymers such as Polypyrrole are favorable for cardiac tissue engineering, owing to its conductivity, easy synthesis, aqueous stability & biocompatibility [2]. Cellulose is a biocompatible bio-polymer with fibrous morphology similar to ECM, high flexibility & mechanical strength, making them ideal as cardiac scaffolds [3]. Here, combining the properties of BC and PPy, we examine BC-Ppy composites as potential scaffolds for cardiac tissue engineering.

The scaffolds were characterized by FT-IR, SEM, TEM, TGA and 4-probe keithly instrument to study their size, structure, morphology and conductive properties. We modified the conductivity of BC-Ppy to achieve conductivity in the cardiac tissue range ($\approx 10^{-3}$ S/cm). Suitability of the scaffolds for cardiac tissue engineering are currently under investigation using cardiac fibroblasts and H9c2 cells for cell attachment, viability, proliferation and cardiomyocyte specific expressions. To the best of our knowledge, BNC-Ppy has not been explored as an effective composite material for as scaffolds for cardiac tissue. In this work, the intrinsic electrical conductivity, excellent biological and mechanical properties is shown to provide favourable environment for the growth and development of cardiac cells.

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Bacterial cellulose membranes loaded with bioactive compounds formulated in neoteric solvents: a joint strategy to improve their biological efficacy

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Abstract

The adequate formulation of bioactive compounds (such as antibiotics, vitamins antioxidants among many others) and their inclusion in efficient delivery systems are essential steps to improve their efficacy. In this context, the formulation of such active principles with neoteric solvents such as ionic liquids (ILs) and eutectic solvents (ES) represents a promising strategy. Furthermore, bacterial cellulose membranes show to act quite efficiently as reservoirs and delivery systems of these bioactive formulations in dermal and also in gastrointestinal applications. Furthermore, the adequate combination with poly(ionic liquids) allow for the design of stimuli responsive (e.g. pH) systems.

A global perspective on the preparation of these formulation, their enhanced biological properties, as well as their incorporations in bacterial cellulose membranes, and results on controlled release will be presented in detail.

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Hydrating patches from bacterial nanocellulose – a platform approach for the treatment of nail diseases

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Abstract

So far, the focus of the use of bacterial nanocellulose (BNC) for medical purposes preferentially lies in its application as a wound dressing, since its unique characteristics including biocompatibility, mechanical stability and high water content are highly beneficial for wound treatment [1]. The topical treatment of nail diseases, such as onychomycosis or nail psoriasis, could also benefit from these properties, since the low permeability of the nail plate for therapeutics can be enhanced by hydration [2]. Therefore, patches based on BNC supplemented with glycerol and urea were developed as a platform for the delivery of drugs through the nail plate.

After static cultivation using *Komagataeibacter xylinus* BNC patches were loaded with glycerol and urea in different compositions with a submerse adsorption technique. The drugs ciclopirox olamine and *Boswellia serrata* extract (BSE) were additionally incorporated to aim for the treatment of onychomycosis and nail psoriasis, respectively. Patches were characterized for their mechanical stability, transparency, as well as their hydrating properties towards keratin films made of human hair, which were used as a nail model. *In vitro* release and drug permeation through keratin films were investigated in Franz diffusion cells.

While the mechanical characteristics of the BNC patches were not influenced by the incorporation of urea and glycerol, an increase of transparency dependent on the amount of incorporated glycerol could be observed. In the hydration experiments, an increase of moisturization towards keratin films as well as a decrease of evaporated fluid loss of the patches could be demonstrated, when comparing to native BNC. A biphasic release from the patches could be observed for both drugs in the Franz cell experiments. A correlation of the glycerol dependent hydration capacity and drug hydrophilicity on the drug flux was demonstrated for the permeation through keratin films.

Conclusively, the feasibility of BNC patches for topical nail applications could be demonstrated. While the hydrating capacity of drug-free patches could be used for the pre-treatment of nails before other therapeutic measures, e.g. the laser therapy of onychomycosis, the permeation enhancement of ciclopirox olamine and BSE demonstrated their applicability in drug delivery for onychomycosis and nail psoriasis. Additionally, the increased transparency paired with the easy applicability of the patches can positively influence patient acceptance and therefore, therapy progress.

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Potential of bacterial nanocellulose in reconstructive surgery of body integumentary system - preliminary studies in animals

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Abstract

The medical application of BC in reconstructive surgery as well as wound healing is still under development, but without proved success of repetitive results. The aim of the study is to present the preliminary results of the in vivo application of *Komagataeibacter xylinum* E25 bacterial cellulose (BC) as a replacement material for produced defects during operations. The use of BC has no adverse effects such as ulceration or extrusion and possesses expected properties. Based on preliminary animal BC fittings are promising implants for various reconstructive applications since they are biocompatible with properties allowing blood flow, attach easily to wound bed and remain in place until donor site is healed properly. BC can be fabricated into patient specific shapes and size, with capability to reach mechanical properties of relevance for heart valve, ear, and muscle replacement. Bacterial cellulose appears to be one of the materials that allow extensive application in the reconstruction after soft tissue defects.

In this study, nine pigs (suf scrofa domestica) were subjected to the same cavities in the ear cartilage (4 x 4 cm) and in the rectus abdominis muscle (6 x 10 cm) with BNC membranes sewn into them. After observing the local and general condition of the animals for 30 days, no clinical signs of complications in the form of inflammation or necrosis in implantation sites were observed. Histologically, a normal scar was produced as a result of the material healing into the host's body. In one case, no residual implant material was found at the site of implantation, and the remodeled scar confirms proper healing. Moreover, no systemic inflammatory reaction was observed in any of the animals.

The host organism's reaction to the implanted bacterial nanocellulose allows us to believe that it meets the expectations as a material that can be widely used in reconstructive surgery. Nevertheless, this requires further research on a larger group and in groups where there will be contact with other foreign bodies. The next step would be to conduct an experiment on a group consisting of people.

Keywords: bacterial cellulose (BC), reconstructive surgery, mechanical properties, biocompatibility and biodegradability

SESSION 4 - MOLECULAR TOOLS AND BIOSYNTHESIS (chairperson: Stanislaw Bielecki)

KEYNOTE LECTURE: Mutant analysis for understanding cellulose biosynthesis in *Gluconacetobacter xylinus*

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Abstract

Over the years, mutants of *Gluconacetobacter xylinus* in which cellulose biosynthesis is affected have been isolated from a number of strains. Generally, mutants affected in cellulose biosynthesis are identified based on their smooth colony morphology and their ability or lack thereof of producing a cellulose pellicle in liquid medium. In addition to spontaneous mutants, mutants affecting cellulose biosynthesis were isolated following chemical mutagenesis, transposon mutagenesis and site-directed mutagenesis. These mutants were useful in isolation of genes in the *bcs* operon, determining the requirement of genes in the *bcs* operon for cellulose synthesis and their possible function, characterization of cellulose synthase, identification of a second cellulose synthase gene, and identification of novel genes that have a role in cellulose biosynthesis. Even as genome sequences of a number of *G. xylinus* strains are available, isolation of more mutants and their analysis will be essential for identifying all the genes that play a role in cellulose biosynthesis in *G. xylinus*.

Size modulation of bacterial cellulose spheroids in *Komagataeibacter xylinus*

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Abstract

Bacterial cellulose (BC) is usually formed by acetic acid bacteria as a pellicle floating at the air-liquid interface in glucose-rich media. On the other hand, under shaking conditions, millimetre-scale round BC particles, named BC spheroids can be produced by some strains. BC spheroids are expected to be used as building blocks for three-dimensional (3D) materials. However, the growth and formation process of BC spheroids has been less well understood, so controlling their size and shape has been a challenge. We focused on BC spheroids as building blocks for 3D food printers. Using *Komagataeibacter xylinus* isolated from fruit vinegar in Nagano, Japan, we attempted to modulate the size of BC spheroids by adjusting the initial concentration of the bacteria cells. The results suggested that there was a correlation between the concentration of the bacteria cells and the size of BC spheroids. Specifically, the BC spheroid size was found to decrease with higher concentration of the bacteria cells; the diameter of the BC spheroid ranged from several hundred micrometres to several millimetres. It was also suggested that the internal structure of the spheroids differed among the strains. At present, only the strain of *K. xylinus* isolated from fruit vinegar was able to reproducibly modulate the size of BC spheroids, and other strains are under the investigation. Furthermore, we plan to examine how effect to the formation of spheroids by the container materials and mixing conditions. This study enables size modulation of BCs on the micro to millimetre order and is expected to be applied as a next-generation BC material.

This work was supported by Moonshot Agriculture, Forestry and Fisheries Research and Development Program (MS508).

Draft genome analysis of new strain of *Komagataeibacter xylinus* isolated from the production environment of fruit vinegar

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Abstract

A bacterium showing the ability of cellulose production has been isolated from the production environment of fruit vinegar in Nagano, Japan. The results of 16S rRNA gene analysis showed 100% match to the type strain of *Komagataeibacter xylinus* DSM 2325 (CP025269.1), L96 (JX283294.1), NBRC 13693 (AB645737.1) and NBRC 15237 (AB680815.1). From this result, it was thought that a new isolate in this time is a derivative strain of *K. xylinus*. A draft genome sequence of new strain of *K. xylinus* was performed in the present study, because a difference was observed when the cellulose production capacity of this strain was compared with that of *K. xylinus* NQ5. The assembled genome had 97 contigs contributing to a total length of 3.7 Mbp with an average G+C content of 62.3%. Predicted coding DNA sequences were 3527, of which approximately 70.4% were assigned the functions. Regarding genes related to cellulose biosynthesis, at least four genes showing homology with the cellulose synthase gene (*bcsA*/*bcsAB*) were confirmed. Among them, only one *bcsA* gene was contained in the *bcs* operon involved in the formation of a typical terminal complex. In addition, two were adjacent to or close to *bcsC* gene, and one was present without the other *bcs* operon-constituting genes. There was one gene encoding phosphoglucomutase and one gene encoding UDP-glucose-1-phosphate uridylyltransferase, which are the supply enzymes of UDP-glucose, which is the substrate for cellulose synthesis. When the homology search of the sequence of each contig obtained by this draft genome analysis was performed, the homology was high as a whole with the genome sequence of *K. xylinus* DMS 2325. The distinction between chromosomal DNA and plasmid DNA and detailed analysis of the sequence are currently underway.
This work was supported by Moonshot Agriculture, Forestry and Fisheries Research and Development Program (MS508).

A synthetic biology approach to growing a photograph

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Abstract

Since the invention of optogenetics two decades ago, researchers have regularly demonstrated the light-responsiveness of their engineered cells by showing a photo of cell culture or a petri dish of bacteria reproducing a projected image such as "Hello World" in fluorescence or luminescence signal. Realising that cellulose-producing bacteria could also be engineered to respond to light via similar methods, we set out on a self-inflicted challenge to engineer *K. rhaeticus* bacteria to not only make such a photo image, but to also make the material it is produced on too. We first introduced into *K. rhaeticus* the DNA encoding an optogenetic system for *E. coli* based on split T7 polymerase, giving us a low-resolution system to induce gene expression in response to blue light in our cells. We used this to produce patterns of red fluorescent protein (RFP) in growing cellulose pellicles. We then went through rounds of optimisation of the genetic construct, culture conditions and light projection system to achieve sub-millimetre scale resolution of image, with tunable RFP levels based on light intensity. Next, as a further challenge, we wanted the grown photograph to be permanent and able to leave the lab. Unfortunately, we found that all common sterilisation methods destroy RFP colour, as well as the colours of several chromoproteins that we can express in *K. rhaeticus*. Therefore, we next introduced expression of a tyrosinase enzyme that catalyses the production of the black pigment, melanin, which is stable after autoclaving and known to fix permanently onto materials and fabric. Optimisation of the genetic construct expressing this enzyme in the cells, and the culture conditions for material growth allowed us to grow cellulose with intense black colouring throughout, that can be sterilised and leave the lab. With genetic systems for image generation and pigment production, it remained for us just to combine these synthetic gene constructs into our cellulose-producing cells to grow our photograph, but so far this has not proven to be straightforward due to genetic and culturing reasons. Tackling this final step has now forced us to develop new approaches to genetic modification of *K. rhaeticus,* including genomic integration for which we've now built standard methods. So, while this whole project may seem quite frivolous, it has proved an excellent focus for us to develop new genetic tools for manipulating *K. rhaeticus* that we now share with the community.

Control of bacterial cellulose biosynthesis guided by systems-level insights

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Abstract

For many decades, cellulose biosynthesis process was mainly studied in terms of improvement of its yield through modification of culturing conditions in the industrially relevant settings. Only since recently, it has been realized that further improvements require deeper understanding of the microbial producers, i.e., their molecular physiology. The majority of the highly efficient cellulose producing strains represent the *Komagataeibacter* genus. Recently, the systemic information for this genus has been accumulating in the form of systems-level datasets representing such omics studies as: genomics, transcriptomics, proteomics and metabolomics [1]. This systems-level data has shown to be useful in guiding studies aiming at the enhanced and tailored cellulose biosynthesis process.

We will discuss the recent findings regarding phylogenetics and comparative genomics of the *Komagataeibacter* species and highlight their common and distinctive features [1,2]. We will further show how transcriptomics explains the distinctive yield and properties of cellulose produced by the bacteria growing in media containing such metabolic enhancers as: ethanol, lactic acid and vitamin C [3-5]. Moreover, we will show how these results drew attention to underexplored regions of *Komagataeibacter* genome which impact the properties of BNC. Our findings improve the understanding of the genotype-phenotype relationship of a cellulose producer, which is important for scaling-up of BNC synthesis and expanding its application [6].

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ORAL COMMUNICATIONS: 23 September Day 2

SESSION 5 - BIOMEDICAL APPLICATION, PART 2 – WOUND DRESSINGS & DRUG DELIVERY (chairperson: Anna Roig)

KEYNOTE LECTURE: Bacterial nanocellulose based materials for dermal applications

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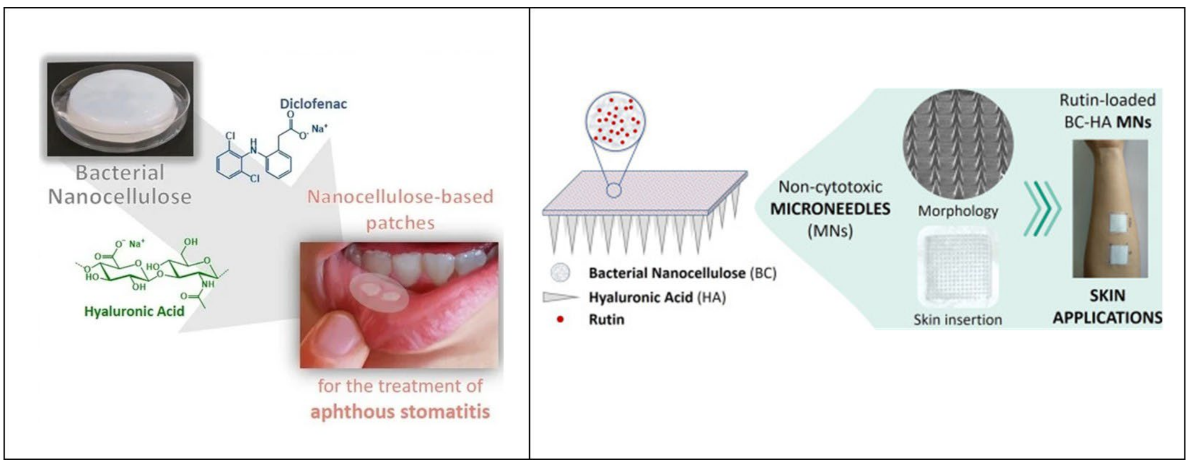
Abstract

Bacterial nanocellulose (BNC) is a unique biobased source of new functional and sustainable materials for a myriad of applications. In particular, the use of this highly pure form of nanocellulose, with peculiar features (nanostructured porous structure, excellent mechanical properties, biocompatibility, among others), in the biomedical, pharmaceutical and cosmetical fields, e.g., as wound healing materials, drug delivery systems and skin care products, has become an active area of research and development.
An overview of the recent research efforts of our group on the design of BNC-based materials for dermal applications will be presented, with focus on the use of BNC to fabricate (i) topical drug delivery patches [1], (ii) microneedles systems [2] and bioinks for 3D-bioprinting of 3D-constructs (e.g. for wound healing).



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Drug Delivery Strategies using Bacterial Nanocellulose: What is next?

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Abstract

Bacterial nanocellulose (BNC) has drawn tremendous attention over the last 10-15 years as drug delivery system due to its unique supramolecular nanostructure and outstanding mechanical, thermal and biological characteristics. Additionally, the automation and upscaling of processes for the controlled production of BNC provided access to high quality material in reproducible quality and opened the avenue toward the application of BNC as drug delivery system.

However, the drug portfolio for loading in BNC revealed a clear preference for hydrophilic (>80 %), small molecules (79 %) with lop P values below 4, preferentially for local applications and treatments with a focus on dermal and transdermal administration with fast release profiles. The potential for the delivery of lipophilic or high molar mass drugs, long-term treatments, more sophisticated or stimuli-responsive drug release profiles, and the establishment of drug delivery strategies beyond wound dressings and skin applications are still offering a huge potential to be explored for BNC.

In the presentation the current status as well as actual limitations of drug delivery applications using BNC as matrix and carrier will be summarized and innovative ideas and progress in the development of more controllable loading techniques, particularly for lipophilic drugs and new applications highlighted.

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Tailoring Bacterial Cellulose dressings to the requirements of chronic wound care

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Abstract

In recent years, hydroactive wound dressings made of Bacterial Cellulose (BC) have become increasingly popular in the treatment of burns. [1–3]. However, although the moist environment provided by the dressing can help to facilitate wound healing in chronic wounds as well, the application on severely exudating wounds can pose a challenge due to its high initial moisture saturation.
A multi-center observation study in 50 patients was performed, evaluating a BC based hydroactive dressing in a chronic wound setting. Subsequently, the dressing was modified to reduce moisture saturation and compared in-vitro to six other commercially available advanced wound care products according with EN-13726 regarding exudate absorption, moisture vapor transmission rate and fluid donation.
The results of the study demonstrate the potential of BC wound dressings to reduce irritation and fibrinous tissue and induce healing even in stalling wounds. Since exudate management of the moist dressing showed room for improvement, a modification of the material was developed showing increased absorption capacity while retaining positive characteristics such as moisture vapor transmission rate and fluid donation capability.
Although further clinical observations are needed to fully explore the potential as well as the limitations of BC in the treatment of chronic wounds of different types and stages of healing, the outcome of this initial clinical study was found to be very promising. Furthermore, a modification of the material with enhanced exudate handling properties could be a suitable candidate in the treatment of chronic wounds with different exudate levels and contribute to a fast and successful wound healing process.

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Multifunctionalization of bacterial nanocellulose for improved wound dressing properties

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Abstract

During the last decades, bacterial nanocellulose (BNC) has become an attractive renewable resource for an increasing number of material applications. In particular, its use in cosmetics (e.g., facial masks), medicine (e.g., wound dressings) and food technology has greatly advanced, employing its inherent high purity, hydrophilicity and physiological harmlessness. While the use of BNC in food technology relies on properties, such as indigestibility for humans (dietary fibers), thermal insulation (retarded melting of ice cream), or stabilization of oil-in-water Pickering emulsions (edible oleofilms [1]), traditional cosmetic and wound dressing applications employ primarily the excellent moistening capabilities of BNC. However, there are far more opportunities if full advantage is taken from the BNC's surface chemistry, hierarchical open-porous morphology and biocompatibility.

In this talk, we present some recent complementary contributions of collaborative work targeting development and production of advanced BNC wound dressing materials for improved patient care. This includes efforts to i) increase the moisture holding capabilities of respective dressings by implementation of percolating alginate networks [2], ii) reduce the stickiness to wounds and sore edges by surface hydrophobization, such as by moisture-induced polymerization of ethyl 2-cyanoacrylate [3], iii) employ their specific morphology for controlled diffusion and release of therapeutic compounds, such as bactericidal polyhexamethylene biguanide [2], iv) use the large internal surface rich in hydroxyl groups for covalent grafting of photosensitizer molecules by aqueous click-chemistry at ambient conditions [4] impart photomicrobicidal properties (gram-positive bacteria, such as *S. aureus* and *B. subtilis;* [5]) concomitant with the capability of destructing malodorous compounds by singlet oxygen and v) develop an fluorophore-based in-situ sensing system for elevated protease concentrations in chronic wounds by grafting a tripeptide motif onto the interface of the wound dressing, capable of triggering a specific color response to elevated concentrations of human neutral elastase [6].

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Elastic Tubular Bacterial Nanocellulose Composites for Small-caliber Vascular Grafts

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Abstract

Bacterial Nano-Cellulose (BNC) tubes can be synthesized *in situ* with special bioreactors [1-3]. Although BNC tube has considerable potential as a small diameter artificial vessel (<6 mm), its poor elasticity, lack of compliance and low suture strength in a hydrogel state, disfavor surgical operations [4-6]. Therefore, the improvement of mechanical properties is important to the transplant effect of the BNC tubes applied as small-caliber artificial vessels. Some synthetic materials such as polyvinyl alcohol (PVA) are polymers with certain elasticity in the gel state [7], and PVA can precipitate in an alkali aqueous solution. In this study, after introduction of PVA into the nanofibril network of BNC tube wall, concentrated alkali mercerization treatment [8] and cryogenic induced phase separation were used to obtain elastic BNC/PVA composite tubes. The potential of the obtained tubes as small-caliber artificial vessels was evaluated by investigating mechanical properties, blood compatibility, cell compatibility *in vitro*, and biocompatibility *in vivo* using a SD rat model. The results would bring a better clinical application of the BNC-based small-caliber artificial vessels.

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Novel Functional Bacterial Cellulose composites used for Tissue Regenaration and Repair

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Abstract

Bacterial cellulose, with unique structural features, physicochemical properties and impressive biological characteristics, is widely used in biomedical fields. In our recent studies, a set of technologies, including self-crosslinking, in-situ composite, in-situ template-growth method, were used to introduce active chemical groups, bioactive molecules, nanoparticles and conductive polymers to BC matrix, so as to achieve its applications in wound healing, nerve repair and urethral repair [1]. A novel composite was prepared by introducing polyethylene glycol and polyhexamethylene biguanidine into BC matrix. The composite exhibited high transparency, water retention ability, flexibility as well as good anti-adhesion property. Moreover, it displayed excellent biocompatibility and strong and sustained antibacterial effect. And it could efficiently promote skin wound healing and regeneration in a rat model. A bifunctional group modified bacterial cellulose was prepared by carboxymethylation and selective oxidation. Further, the chitosan was compounded in the network of DCBC by self-crosslinking to form dialdehyde carboxymethyl bacterial cellulose/chitosan composite. The composite exhibited excellent antibacterial activity and promoted proliferation and migration of HUVEC. Moreover, it could accelerate healing of deep Ⅱ degree scald wounds of Bama miniature pig through epidermal growth and collagen production [2]. A multiblock conductive nerve scaffold with self-powered electrical stimulation was prepared by in situ polymerization of polypyrrole (PPy) on the nanofibers of BC. Platinum nanoparticles were electrodeposited on the anode side for glucose oxidation, while nitrogen-doped carbon nanotubes (N-CNTs) were loaded on the cathode side for oxygen reduction. The scaffold showed good mechanical property, flexibility and conductivity. Dorsal root ganglions cultured on the scaffold showed significantly longer neurite outgrowth than those on the BC/PPy group. And the scaffold promotes nerve regeneration of rats. BC/poly(3,4-ethylenedioxythiophene)-sulfonated nanofibers composite was prepared through the in-situ polymerization of PEDOT and the doping of SNFs in BC matrix. The polymerization of PEDOT endowed BC with conductivity, making the membranes conducive to the adhesion and proliferation of adipose-derived stem cells. The membrane exhibited great biocompatibility, high conductivity , and greatly improved the peripheral nerve repair of rats. Strength [3]. A double-modified bacterial cellulose/soybean protein isolate (DMBC/SPI) using as urethral tissue engineering scaffold was prepared. The scaffold displayed good biocompatibility and biodegradability. In vitro, DMBC/SPI promoted cell adhesion, cell proliferation, and guided directional growth of stem cells. In vivo, it enhanced the urethra tissue repair and did not cause a inflammatory response.

These studies show the functional diversity of BC composites and the bright prospect of clinical application in human body.

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SESSION 6 – FERMENTATION (chairperson: Tetsuo Kondo)

Bacterial Cellulose production: valorization of wastewater and Life Cycle Assessment

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Abstract

Low-cost substrates, most from agro-industrial wastes, have increasingly been exploited as nutrient sources for the fermentation of bacterial cellulose (BC), an appealing approach from an economical and environmental point of view. However, these wastes carry a very high organic load, which, while advantageous for the fermentation, generate high organic load wastewaters as well, which require proper treatment before the release or recycling of the treated water, which may have a significant impact in the economic and environmental sustainability of the BC production.
Anaerobic digestion (AD), a process that produces biogas (primarily a mixture of methane and CO2) typically used for lighting and heating, is one of the most appropriate and promising treatments for high loaded industrial wastewaters. In this work, wastewaters from BC fermentation were characterized, as well as their biochemical methane potential and anaerobic biodegradability. The performance of an upflow anaerobic sludge blanket reactor (UASB) for the treatment of these wastewaters was also evaluated. Briefly, A relevant among of biogas could be produced from AD, while reducing the chemical oxygen demand (COD, an indirect measure the amount of organic compounds) of the treated waters [1].
The Life Cycle Assessment (LCA) is a methodology used to quantify the environmental, health and resource depletion impacts related to products, processes, and services. A LCA was used to a projected production of BC under static culture, including wastewater treatment, following a cradle-to-gate approach. From this study, a considerable amount of water is consumed, most of which being treated and emitted to the environment (to fresh water). The BC production facility itself had a small contribution to the consumption of resources and environmental impact of the global life cycle, most of which were associated with the production and transport of materials. Further, a comparative LCIA was made against plant celluloses. Briefly, with the increasing environmental awareness, BC production may be a strong candidate towards the reductions of environmental impacts and risks, concerning climate change and fossil resource depletion, while providing a viable, economically and environmentally sustainable bioproduct, with unique properties for a wide range of market applications [2].

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High-yield Production of Nanofibrillated Bacterial Cellulose (NFBC) in Aerated and Stirred Bioreactors

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Abstract

Nanofibrillated bacterial cellulose (NFBC) is a type of cellulose nanofibrils (CNF) directly obtained from *Gluconacetobacter* (*Gluconacetobacter intermedius* NEDO-01) culture under agitation in the presence of water-soluble polysaccharides as dispersants [1]. Being produced through a bottom-up process, NFBCs show advantages as reinforcing fillers over other CNFs prepared through top-down processes: e.g. extremely long fiber length (> 15 μm), tunability of the dispersibility in water or several polar organic solvents depending on the selection of dispersing agents (carboxymethylcellulose, hydroxyethylcellulose, or hydroxypropylcellulose) [2] as well as the post-production surface modification with silane coupling agent [3].

At first, the production rate of NFBC was insufficient (< 1.0 g/L/d) for industrial production. Therefore, we have investigated the effects of several production conditions on the productivity and the characteristics of NFBCs generated in aerated and stirred bioreactors. The pH adjustment using NaOH during culture periods significantly increased the production speed of NFBC (1.8 g/L/d). On the other hand, by pressurizing the culture, the production speed increased more (2.0 g/L/d). By combining these methods, the final production speed was increased to 3.75 g/L/d. The material characteristics of NFBCs generated under these production conditions showed no significant difference compared to those obtained by the control method (without pH adjustment nor pressurization) using a conical flask with baffles.

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Bacterial nanocellulose production from carbon dioxide

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Abstract

Carbon dioxide, the primary greenhouse gas emitted by human activity, is the pollutant that most influences global warming and the consequent climate change. CO_2 capture and utilization, and when possible its further valorization, is proposed as a powerful technological strategy to alleviate the problem of greenhouse gas accumulation. We have isolated a bacterial strain able to produce cellulose when growing with naphthalene as sole carbon source [1, 2]. The strain belongs to the sulphur oxidizing bacteria and can also use a number of compounds and residues as carbon source to produce cellulose. We show that the strain can grow and produce cellulose when growing with carbon dioxide as sole carbon source and reduced sulphur compounds as source of reducing power. The cellulose biofilm is produced in thin parallel layers under static conditions, and forms ball-shaped aggregates at different shaking speeds. The produced polymer has all the characteristics of bacterial nanocellulose, namely 60 nm wide - several µM long fibres, with a typical cellulose FTIR spectrum. Analysis of the strain's genome identified a *bcs* cluster for cellulose synthesis. Knock-out mutants in *bcsA* or *bcsK* genes were capable of growth with carbon dioxide, but did not produce cellulose. The genome included the gene complements for a complete Calvin-Benson-Bassham cycle for CO_2 fixation and for two pathways (*sor* and *sox*) for sulphur compound oxidation. However, a *cbbL* mutant in the RuBisCO large subunit gene was still capable of growth with carbon dioxide, suggesting the functioning of an alternative carbon fixation pathway. A spontaneous mutant with an increased capacity of cellulose production was isolated. We identified a large chromosomal deletion in the mutant genome that included, among others, the genes for a quorum sensing regulation system. Site-directed knock-out mutants in different genes of the QS system also showed increased levels of cellulose biosynthesis. The optimum conditions for cellulose production from CO_2 are being established. The bacterial nanocellulose produced can be mechanically functionalized, with the potential for CO_2 capture to reach levels above those presented for other conventional and non-renewable materials.

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Bacterial nanocellulose-based composites as eco-innovative materials with potential industrial and medical significance

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Abstract

Microbiologically synthesized cellulose (bacterial cellulose, BNC) is a natural and remarkably versatile biopolymer, exhibiting high chemical purity and extraordinary properties that can be utilized either in its native state or in combination with other compounds to alter its structure and function. Currently, the tailored design of novel green composites is one of the most dynamically developing trends in the field of modern materials science and engineering. The targeted BNC modification opens up new opportunities for its utilization in a wide range of applications, from more established ones, such as medical (e.g. drug delivery carriers, wound dressings, tissue engineering scaffolds, implants), through biotechnological, agricultural, environmental (e.g. water/wastewater treatment, pollution detection, filtration materials), up to more innovative ones, such as electronic (e.g. sensors, energy storage and transfer devices, acoustic membranes, OLEDs) and active/intelligent food packagings [1-3].

The aim of the research was to obtain multifunctional bacterial nanocellulose-based composites with various commercially attractive features. Herein, bacterial nanocellulose was modified at the stage of its biosynthesis (i.e., *in situ* method) by supplementing the growth medium with three representative polymeric additives, such as citrus pectin (CP), polyvinyl alcohol (PVA), and polyethylene glycol (PEG). This one-step strategy allows the compounds to become part of the growing cellulose fibril network, resulting in stable bio-inspired composites. Subsequently, the obtained BNC films were subjected to a detailed comparative analysis in order to assess the impact of polymeric additives on the selected properties of the nanocellulose matrix, as well as the possibilities of their potential application.

The results showed that selected compounds significantly changed the physico-chemical and mechanical properties of chemically inert bacterial nanocellulose, especially the dye loading abilities (BNC/CP), tensile and compression strength (BNC/PVA, BNC/PEG), and water adsorption/retention capacities (BNC/PEG). Thus, the obtained bio-inspired composites can be potentially useful in various fields of industry and medicine, and in the future, they may become a valuable and cost-effective competitor against commercial materials currently available on the market.

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SESSION 7 - COMPOSITES AND APPLICATIONS, PART 2 (chairperson: Dieter Klemm)

Reinforced natural hydrogels of collagen and bacterial nanocellulose for 3D culture of mesenchymal stromal cells

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Abstract

There is a growing interest in the development of hydrogels derived from natural materials to culture cells in 3D scaffolds mimicking their *in vivo* microenvironment. Hydrogels obtained from animal tissues are promising however, they usually exhibit suboptimal mechanical properties in comparison to the native tissues to mimic. Thus, this work aims to stiffen type I collagen hydrogels with bacterial cellulose (BC) fibers to develop scaffolds with tunable mechanical properties suitable for 3D cell culture. BC fibers were produced from BC pellicles biosynthesized from *Komagataeibacter xylinus* and mixed with high concentrated type I collagen obtained from tendons of rat tails. Laser confocal microscopy and scanning electron microscope images confirmed the homogenous macro- and micro-distribution of the two types of polymers in the hydrogel. Tensile strength and rheology measurements demonstrated the reinforcing effect of BC fibers by a 43 % increase in the stiffness for the composite hydrogel while maintaining the same viscoelastic response. Biocompatibility was tested by culturing human bone marrow-derived mesenchymal stromal cells within the reinforced hydrogels for 7 days; cells showed good viability, proliferation and adhesion to the scaffolds. These novel hydrogels of collagen reinforced with BC fibers might emerge as useful platforms for 3D *in vitro* organ modeling, tissue engineering applications as well as for mechanobiology studies.

Bacterial cellulose is alive': Practitioners' experiences developing bacterial cellulose as a versatile emerging biomaterial

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Abstract

Purpose – Bacterial cellulose has developed extensively within the last few decades as a versatile biomaterial for application in several industries. While scientific research into the material continues to advance, there is little research investigating its uptake in industry from the perspectives of practitioners. This interdisciplinary research project discusses the trends, challenges, and opportunities regarding bacterial cellulose as a material to provide rich insights to guide study and contributions within future bacterial cellulose academic literature.
Study methodology/approach – The study takes a qualitative approach through analysis of 10 semi-structured interviews conducted with sustainable small-medium enterprises, material company producers, designers, scientists, and researchers to investigate the trends, challenges and opportunities that arise with bacterial cellulose amongst a wide range of material users.
Findings – This study uncovered the motivators that drew people to pursue research and practice in bacterial cellulose, the characteristics sought from bacterial cellulose as an emerging material, advantages and limitations that will drive the future trajectory of bacterial cellulose, as well applications and recommendations for bacterial cellulose's future commercialization and industry use. The study highlights practical insights on the barriers for greater uptake of bacterial cellulose such as challenges to scale and lack of accessible knowledge, as well as personal perspectives from practitioners on their relationship with the material and its potential. Observing how bacterial cellulose practitioners share similar yet differing experiences and recognizing the reflective practice of such experiences as crucial within the scientific literature can help develop bacterial cellulose as a sustainable biomaterial for many applications.
Originality/value – This is the first study to apply qualitative content analysis to better understand the perceptions and realities of the current bacterial cellulose material in development. Responding to the lack of in-depth discussions regarding bacterial cellulose's future identity as a material in the scientific literature, this study developed an in-depth understanding of the reasons why a range of people including small-medium enterprises, material companies, designers, scientists, and researchers engage with bacterial cellulose from many industries. Although bacterial cellulose may not be currently feasible for commercial-scale manufacturing, the study provides key insights for future research based on practitioner perspectives.

Keywords: Bacterial cellulose, Biomaterials, Biotechnology, Discussion, Interviews, Practitioners, Qualitative Content Analysis, Sustainability

Bacterial Cellulose Nanofibrils as a Novel Vaccine Adjuvant

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Abstract

Bacterial cellulose (BC) is an unbranched biopolymer produced by microorganisms, composed of glucopyranose units linked by β-1,4 bonds [1], [2]. In this study, adjuvant action of BC nanofibrils has been investigated *in vitro* using bovine serum albumin (BSA) as model antigen. BC produced by static culture of *Komagataeibacter xylinus* was microparticleated by acid hydrolysis [3]. BC nanofibrils were then conjugated with BSA and characterized using Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) [4]. Subsequently, cytotoxicity, TNF-α and IL-6 cytokine secretion and cellular uptake of BC nanofibrils conjugated with BSA on the human monocytic cell line (U937) differentiated into macrophages were tested [5]. The highest TNF-α cytokine level has been obtained with BSA-conjugated BC and it was statistically significant compared to the positive control group (BSA-alum), while IL-6 cytokine levels obtained the same as in the control group. BC nanofibrils were capable of increasing the immunogenicity of antigens by inducing cellular uptake and TNF-α secretion and showed high viability in macrophage cells. These results demonstrate for the first time that BC has the potential to serve as a vaccine adjuvant.

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Development of layered BNC composites for Food Packaging

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Abstract

The food industry is increasingly demanding advanced and sustainable packaging materials with improved physical, mechanical and barrier properties. The currently used materials are synthetic and non-degradable, which raises environmental concerns. Research efforts have been made in recent years towards the development of bio-based sustainable packaging materials. One of those is nanocellulose, which have a potential to be used as matrix, as nanofillers or as coatings for composites [1].

A promising material is bacterial nanocellulose (BNC), a biopolymer extruded by *Komagaebacter xylinus* as a 3D nanofibrillar network. BNC offers interesting properties such as high porosity, biocompatibility, non-toxicity and biodegradability [2]. From a food packaging perspective, BNC has a great potential due to the great mechanical performance. However, the high water affinity of BNC is ta major obstacle for food packaging applications [3]. Therefore, the first task was to develop a layered biodegradable composite based on a plasticized BNC (either with glycerol or polyethylene glycol) and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), as an attempt to improve the water vapour permeability. The PHBV coating on plasticized BNC reduced significantly the water vapour permeability (from 0.990 to 0.032 $g.\mu m.m^{-2}.day^{-1}.Pa^{-1}$), increased the hydrophobicity (contact angle from 10-40° to 80-90°), but decreased the stiffness (from 3.1 GPa to 1.3 Gpa) of the BNC composite. The mechanical and barrier properties of the obtained layered composite were considered suitable for food packaging applications.

Although the results obtained being important for food packaging, its commercial use is still far off due to production costs and low production capacity, especially when compared to plant-based nanocellulose [1]. Nevertheless, BNC is a proven material to support substances that play an active/intelligent role in food packaging, with ability to carry and release active substances [4, 5]. Therefore, a functionalized BNC film was developed, by *in situ* incorporating zinc oxide nanoparticles (ZnONPs). The synthesis of ZnONPs was based on co-precipitation method, using zinc acetate and sodium hydroxide (NaOH) (added dropwise) as reactants. In order to prevent aggregation of ZnO NPs, polyvinyl alcohol (PVOH) was used as capping agent.

Overall, dropwise addition of NaOH in zinc acetate-PVOH (with immersed BNC), allowed the production of ZnONPs (≈144 nm), with low polydispersity index (≈0.139) and a homogeneous distribution of ZnONPs on the BNC. Concerning the antimicrobial activity, the minimum ZnO dosage for antimicrobial activity was 20%m_{ZnO}/m_{BNCZnO}, being effective on gram – bacteria (such *Escherichia Coli*) but only on some gram + bacteria (such *Staphylococcus Aureus*). The migration of ZnO onto food simulators are under testing.

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Use of functionalized bacterial cellulose/fibroin as conformable substrate in organic field-effect transistors (OFETs)

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Abstract

Recently, great effort has been made to develop both flexible and conformable electronic devices, either as a way to improve ergonomics, making them fit the human body or even to prevent them from breaking under pressure [1]. The search for cheap, sustainable and, mainly, biocompatible materials becomes necessary, since such devices would be subject to long exposure to living tissues [2]. So far, together with the Organic Light-emitting Diodes (OLEDs) technology and Organic Solar Cells (OSC), there is a demand for new and efficient organic field-effect transistors (OFETs) on biocompatible substrates to allow conformable organic electronics.

Among the materials that can be used for this purpose are bacterial cellulose and fibroin [3], which is a natural biocompatible material extracted from the silkworm cocoon. Here, cellulose was processed and functionalized using fibroin in order to increase the substrate's transparency, while cellulose makes fibroin more malleable and robust. Some of these substrates have also been treated with methanol, which makes fibroin water and moisture resistant [4].

Then, OFETs were fabricated with top-contact bottom-gate architecture, with Al as gate electrode, PU or PMMA (reference device) as gate dielectrics, P3HT as organic semiconductor and, finally, Au as source and drain electrodes.

From the output and transfer curves it was possible to extract the mobility, the threshold voltage and the ON/OFF ratio of the devices. The results for bacterial cellulose treated with fibroin and the fibroin+MeOH were compared to a reference device built onto glass substrate, presenting mobilities of 7.9×10^{-3} and 2.3×10^{-3}cm2/Vs, threshold voltages of 4.0 and 9.7V and ratios ON/OFF of 4.3 and 2.2, respectively. It's quite clear that mechanical stress of the substrates due to the manufacturing processes as well as the manipulation during the measurements affects the performance of the devices.

However, such preliminary results show that these substrates can be successfully used as a platform for the fabrication of conformable and biocompatible OFETs. Here, the main challenge is to improve the manufacturing process, which is complex, since malleable substrates do not support themselves, and the development of a structure more suitable for these substrates, making the devices robust enough to withstand future mechanical stress tests.

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Argon Plasma-Modified Bacterial Cellulose Filters for Protection Against Respiratory Pathogens

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Abstract

Due to the global spread of the SARS-CoV-2 virus and the resultant pandemic, there has been a major surge in the demand for surgical masks, respirators, and other air filtration devices.1 Importantly, the SARS-CoV-2 pandemic has also led to a renewed recognition of the danger posed by airborne transmission of respiratory pathogens,2 meaning that the demand for air filtration materials, whether for personal protective equipment (PPE) or indoor air handling need to remain high. Unfortunately, the fact that currently used filters are made of petrochemical-derived, non-biodegradable polymers means that the surge in production has also led to a surge in plastic waste. Therefore, there is a clear and urgent need to develop novel filter materials with sustainability in mind, with the ultimate goal of facilitating a circular economy in this product area.3 Ideally, such filters would consist of biodegradable materials that can be obtained sustainably, from renewable resources. One example of more sustainable filter material is bacterial cellulose (BC).

In the current study, we present novel, sustainable filters based on bacterial cellulose (BC) functionalized with low-pressure argon plasma (LPP-Ar). The “green” production process involved BC biosynthesis by Komagataeibacter xylinus, followed by simple purification, homogenization, lyophilization, and finally LPP-Ar treatment. The obtained LPP-Ar-functionalized BC-based material (LPP-Ar-BC-bM) showed excellent antimicrobial and antiviral properties, with no cytotoxicity versus murine fibroblasts in vitro. Further, filters consisting of three layers of LPP-Ar-BC-bM had >99% bacterial and viral filtration efficiency, while maintaining sufficiently low airflow resistance (6 mbar at an airflow of 95 L/min). Finally, as a proof-of-concept, we were able to prepare 80 masks with LPP-Ar-BC-bM filter and ~85% of volunteer medical staff assessed them as good or very good in terms of comfort. We conclude that our novel sustainable, biobased, biodegradable filters are suitable for respiratory personal protective equipment (PPE), such as surgical masks and respirators.

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SESSION 8 - INDUSTRIAL LANDSCAPE (chairperson: Miguel Gama)

KEYNOTE LECTURE: Closing the gap to profitable commercialization of bacterial cellulose-based technologies: established large-scale bacterial cellulose production and product form optimization

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CP Kelco U.S., Inc

Abstract

This communication will address industrial large-scale production of bacterial cellulose (BC) and the availability of BC in various forms to support a range of applications and production process of finished goods across industries. CP Kelco has been pioneering high yield submerged fermentation and efficient recovery since it entered BC production in the 1990s. Recent advances in downstream processing (DSP) have allowed further production optimization and debottlenecking of CP Kelco BC production capacity. The idea that BC is not readily available in large, standardized quantities has however survived to this day[1]. It likely stemmed from different terminologies and end-use focus between the academic and industrial communities. This lack of visibility has probably hampered a broader adoption of BC and has given the impression to potential BC users that they needed to start their own “garage production” to access this versatile material.

The context of animal and synthetic ingredients replacement has driven an increasing interest in BC from start-ups and established companies in fields as diverse as personal care, cruelty and synthetic free textile, composite materials, and industrial slurries [2].CP Kelco has developed a range of products including ready to use stable BC dispersions to address various manufacturing process requirements and end use properties of these industries. Working with these industries and companies of various size, we have found that BC produced by submerged fermentation can be suitable for both liquid and solid-state applications. Contradicting the findings from model systems study, BC users did not report significant differences in mechanical properties between static and submerged fermented BC in complex solid-state matrices [3].

The key for industrial adoption of BC is to find the product form that reaches the optimum balance between ease of use and affordability for each application or process. Cellulose content, dispersibility and fibre surface charges are among the main elements that can be adjusted. Optimization of BC products from existing submerged fermentation production capacity allows industrial users of BC to access large, cost-effective supply and avoid unnecessary capital expenditure or cash burn in the case of pre or early revenue companies. CP Kelco has both the production capacity and know-how to partner with current and future BC users even for high tonnage applications.

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KKF – polymers for life: Biotechnologically designed bacterial cellulose materials with application-oriented shape, surface and network structures

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Abstract

In this presentation the biotechnological company "KKF – polymers for life" is introduced. The company KKF was founded in 2005 by a spin-off of scientists from Jena University. The core competence of our research is the development of novel and innovative products in the field of synthetic and natural polymers – especially bacterial cellulose (BC) – together with partners from industry, universities, clinics and scientific institutions.

Our unique selling point is the biotechnological design of BC materials and their characteristics using a dynamic and template-based technology, called Mobile Matrix Reservoir Technology (MMR Tech). The MMR Tech enables to influence the three-dimensional shape. A typical example is the production of BC hydrogel tubes (inner diameter of 5 mm, length up to 175 mm, wall thickness of 1 mm), which withstand inner pressure values >1100 mbar. These characteristics make our BC tubes particularly suitable for small diameter blood vessel replacement. In addition to this high mechanical stability, the design of BC surfaces is also important for the use as an implant material, such as the replacement of other hollow organs in regenerative medicine. Using MMR Tech, the surface structures can be modified by variation of the process parameters and the template materials. Controlled moving of the template between the culture medium and air space allows the construction of different nanofiber network architectures. In addition to a homogeneous structure, the network of BC hydrogels can be created as differently structured multilayers. Designed nanofiber networks are of importance for the development of wound dressings and the incorporation of active substances. Further features of the MMR Tech are the *in situ* production of BC composites by adding dissolved or dispersed additives to the culture medium as well as direct coatings of different types of materials such as papers and textiles with BC. These opportunities afford the development of smart packaging materials and light-weight electronic devices.

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Uncover the untapped potentials of fermented (bacterial) cellulose: application for coatings and cosmetics

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Abstract

Cellugy has developed a method to isolate high biocellulose producing strains All the strains were isolated from a naturally occurred biocellulose-formed symbionts. From several isolates in Cellugy portfolio, one *K. xylinus* species in particular produces high amount of biocellulose reproducibly. Based on the phylogenetic data from whole genome sequencing, our special strain was placed in two distinct clades, diverged from common *K. xylinus* strains supplied from culture collections institute (DSMZ/ATCC) or isolated from vinegar. Unlike other strains[1] Cellugy's *K.xylinus* is capable of producing cellulose in sucrose containing minimal synthetic medium without addition of amino acid, vitamins or trace mineral in agitated-fermentation condition.
As a result, Cellugy's biocellulose has unique structure of long and thin fibrils pristine surface chemistry which can be formulated to different applications. Our biocellulose long and thin fibrils network shown to have high gel strength, superior for thickening ability with emulsification and particle stabilization property. The pristine surface is owing to its high crystallinity, up to 94% (compared to crystallinity of 60% for commercial plant MFC)[2,3], ensuring minimal amount of surface-exposed hydroxyl groups. Long pristine crystalline surface opens new possibility for modification without chemical reactions. We have explored formulation of our biocellulose to Triple Action Coating for fiber-based material (cellulose-based paper or textiles) as oxygen, water, and grease barriers, without any chemical modification, replacing fluorine-based petrochemical coating. Coated material is intended to be able to be recycled together or home composted.
The pristine surface of our biocellulose also makes it stable and less responsive to salts and cationic surfactants, commonly used in the personal care formulation. This is currently an under-explored feature that could broaden the applicability of our biocellulose in complex and challenging formulations with ionic compounds or at low pH. Our pristine and high crystalline fibers open possibility to be made as redispersable powder, easily by modulating its surface to prevent the fibers to collapse when dried. Our biocellulose redispersable powder can be made to be anionic or cationic depending on the modification made before drying process.

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A new generation Bacterial Cellulose based Hydrocolloid for Food Industry

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Abstract

For the first time since industrial animal agriculture started 60 years ago, alternative plant-based diet is gaining attraction and coming close to meeting global demand for sustainable food sources. The popularity of plant-based meat and dairy products – that not only looks, cooks, and tastes like conventional products but also has equal nourishment in protein, fat, vitamins, minerals, and water is the main ask of the consumers.

Food Formulators are scouting for multifunctional ingredients which can help them to design and deliver these traits. The major ingredients apart for protein and fat sources are the texturizers, emulsifiers, aroma, flavors, and colorants etc. The additives like methyl cellulose, xanthan gum and carrageenan, etc are the usual choice available. However, they come with their negatives, forces food formulators for better alternatives to develop a clean label product.

Bioweg addresses this problem with a single ingredient which has multiple functional properties. It thickens, emulsify, stabilise, impart flavors and color to the formulations. In addition to this, it also adds dietary fibre to the final formulation without any additional calories. The major constituent in producing this product is the addition to our proprietary strains of Komagataeibacter and Molasses from sugar industry. We have developed various techniques to sterilise and stabilise molasses in both upstream and downstream processes.

We have deployed mechanochemical methods to impart functionality to the fermentation derived cellulose. We are currently working on enhancing the flavor and color profiles to fit this ingredient to various food applications.

The Hylomorph journey from bench to bedside

Aldo Ferrari

Hylomorph AG, Switzerland

Abstract

Hylomorph is a medical device start-up company based in Zurich, Switzerland. At Hylomorph we aim at minimizing post-surgical complications in medical implants, thus improving patients' quality-of-life and reducing associated healthcare costs. Our core technology, the CellSense, implements antiadhesive microtopography on the surface of bacterial cellulose substrates, empowering them with enhanced antifibrotic properties. The CellSense cellulose enforces an effective and durable protection around target implants. Our first product, the Hylomate envelope, has been developed for the use in combination with cardiac rhythm management devices (CRMDs). Our journey has started back in 2014 in the laboratories of the ETH Zurich and has led us to the first in man application, the Hylomate clinical trial in CRMD recipients, already in 2019. It is now moving toward market approval and additional applications of our unique solution.

KEYNOTE LECTURE: From lab to market – How to build up a successful business?

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Abstract

Within only a few years, JeNaCell developed from a science-based start-up to a certified medical producer of pioneering products for burns and other skin injuries with a production capacity in ton-scale – all based on biosynthetic cellulose (BC). How did this happen?
JeNaCell is specialist for the development and production of BC with a defined shape and controlled 3D structure design. Our proprietary technology for a highly controlled production of planar BC, brand name biocellic®, is the manufacturing base and core element of our company success. With this efficient process, we are able to produce BC, both in large quantities and in consistently high medical-grade quality. In this presentation, we will share our experiences gained during the last years of business start and growth and discuss hurdles and chances we met on our way as a young biotech company. Furthermore, we will share latest developments obtained within our group and together with cooperation partners as well as our prospective on the future potential of the fascinating biomaterial BC.

POSTERS

P1: *In situ* formation of polymeric particles in bacterial nanocellulose for drug delivery using sustainable solvents

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Abstract

Reports on drug delivery from wound dressings based on bacterial nanocellulose (BNC) mostly describe the incorporation of hydrophilic substances by immersion in aqueous solutions. Since this approach is not feasible for poorly water-soluble drugs, their incorporation is usually based on the exchange of bound water with other solvents [1]. These strategies limit the hydrating capabilities of the material, which are essential for wound treatment, and typically lead to a fast release of the incorporated drugs, making frequent dressing changes necessary. To enable the incorporation of lipophilic drugs while remaining the beneficial moisturizing capabilities of BNC and additionally realizing a prolonged drug release, a strategy based on *in situ* phase inversion of poly(lactic-co-glycolic acid) (PLGA) solutions was investigated.

BNC fleeces were produced by static cultivation of *Komagateibacter xylinus*. To identify a suitable carrier to incorporate PLGA, the conventional solvent N-methyl-2-pyrrolidone (NMP) as well as the "green" alternatives polyethylene glycol 400 g/mol, Cyrene™ and ethyl lactate were used to dissolve the polymer prior to loading into BNC with a submerse adsorption technique. Afterwards, a solvent exchange led to the spontaneous formation of PLGA particles within BNC. The solvents were evaluated based on their ability to homogenously incorporate drugs. Additionally, the effect of the loading procedure on the mechanical stability, water binding characteristics and the transparency of the material was evaluated. The suitability of this formulation approach was investigated by additionally incorporating the highly lipophilic drug cannabidiol and determining the release kinetics from the material in Franz diffusion cells.

NMP as well as the "green" alternative ethyl lactate were identified as suitable solvents for the phase inversion, leading to the *in situ*-formation of spherical microparticles with a narrow size distribution that were homogenously distributed within the BNC network. While the precipitation of polymer particles led to a decrease in transparency, the favourable mechanical and water binding characteristics of BNC were not relevantly affected. The encapsulation of cannabidiol in the PLGA particles was possible resulting in an almost linear prolonged drug release over up to 10 days.

The formation of polymer particles within BNC to encapsulate lipophilic drugs is a viable strategy to achieve a homogenous incorporation and prolonged release without negatively affecting BNC beneficial characteristics while minimizing the environmental impact due to the applicability of the method with sustainable solvents. The possibility of polymer inclusion into BNC opens up a broad field of possible applications for drug delivery, e.g. stimuli-controlled release of drugs.

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P2: Modification of bacterial nanocellulose with bioactive conjugates towards burns dressing application

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Abstract

Bacterial nanocellulose (BnC) possesses unique properties that prove its usefulness in the field of burns, such as high water holding capacity (more than 100 times its dry weight), high biocompatibility, high degree of polymerization, high crystallinity (up to 90%), high mechanical stability, non-toxicity and ultra-high purity[1, 2]. In addition, BnC shows high-temperature stability, which allows the process of steam sterilization. Furthermore, its shape can be adapted for contact with tissue. The major limitation of native BnC is its lack of bioactivity (antimicrobial, anti-inflammatory), essential for second-, third- and fourth-degree burns. Several strategies have been developed to induce BnC bioactivity with antibiotics, inorganic components, chitosan, and cationic antiseptics. However, it is necessary to investigate the use of new bioactive substances with a lower tendency to develop antimicrobial resistance, with selective removal of debridement (removing only necrotic, non-viable tissue), faster and more efficient skin regeneration, which will preserve the beneficial properties of BnC and conforms fully to green chemistry principle. We focused on bromelain – a proteolytic enzyme derived from all parts of pineapple with numerous therapeutic efficiency useful in burns, including proteolytic, anti-inflammatory, antibacterial, and anti-edematogenic effects[3].

In this study, BnC membranes were produced extracellularly by a novel species of aerobic acetic acid bacterium *Komagataeibacter melomenusus* via a static fermentation process and purified by alkali treatment (0,5% w/v NaOH solution). Obtained BnC membranes were modified by optimized, bioactive conjugates composed of carboxymethyl cellulose (CMC) and bromelain. The effectiveness of the incorporated bioactive conjugates and their effect on the physico-chemical properties of the BnC membrane were evaluated using a variety of techniques, including *scanning electron microscopy* (SEM), optical microscopy, attenuated total reflection Fourier transform infrared spectrophotometry (ATR-FTIR), thermogravimetric analysis (TGA) and X-ray diffractometry (XRD).

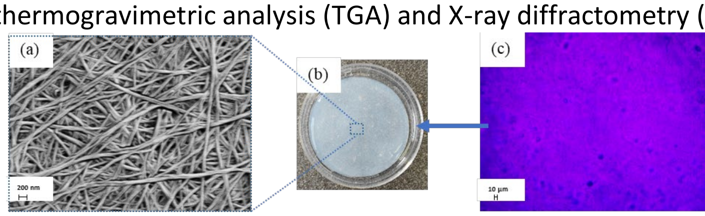

Figure. (a) SEM image of BnC, (b) BnC membrane harvested from static fermentation, (c) optical microscope image of bioactive conjugates CMC-bromelain.

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P3: Addition of sorbitol for the obtention of redispersible bacterial nanocellulose powders

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Abstract

The obtention of powdered products through dehydration of both bacterial nanocellulose (BNC) and plant-based nanofibrillated celluloses is known to lead to the aggregation of the nanofibers and, consequently, the loss of their nanometric size and distinctive properties. This phenomenon, known as hornification, occurs due to the formation of hydrogen bonds among cellulose nanofibrils during the drying process [1]. Given the negative economic and technical implications of the storage, transport and/or use of cellulose nanofibrils as aqueous suspensions, as well as the impossibility of reconstituting the dehydrated powders while maintaining their original characteristics; in recent years various alternatives have been proposed to produce redispersible powders, most of them by addition of chosen capping agents [1, 2].

In this context, with the hypothesis that a polyhydroxylated additive will achieve a disruptive function of the interchain hydrogen bridges by establishing interactions with cellulose hydroxyl groups, the inclusion of sorbitol (a biodegradable, abundant, cheap glucose derivative widely used in the food industry) during the drying process is proposed. The effect of three sorbitol:BNC mass ratios (i.e. 1:1, 3:1 and 5:1), two drying methods (freeze- and oven-drying), and two redispersion strategies (Ultra-Turrax and mechanical stirring) on the redispersibility of BNC powders was studied by means of sedimentation assays and Congo Red specific surface area determination [3].

In agreement with previous results [4], the assays performed indicated that in all cases freeze-dried samples showed better redispersibility than oven-dried ones. Besides, irrespectively of the mass ratio used, the addition of sorbitol prior to freeze-drying led to redispersed BNC suspensions that resembled the never-dried ones. The benefits derived from sorbitol addition were even more noticeable for oven-dried powders. Contrary to freeze-dried samples, oven-dried samples without sorbitol addition did not redisperse at all. On the other hand, oven-dried additivated powders could be redispersed in water, with much better results as higher sorbitol:BNC ratios were used.

All these results, together with the possibility of easy removing sorbitol by proper filtration, suggest that its addition could be a promising strategy to produce redispersible BNC powders.

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P4: Bacterial cellulose a natural hydrogel for the treatment of ocular surface and anterior segment pathologies

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Abstract

The cornea is the outermost part of the eye and is constantly exposed to physical, chemical, and biological stressors. Many pathologies require the administration of drugs, and those pharmaceuticals are delivered to the anterior segment of the eye via eye drops. However, the efficacy of an eye drop is limited due to its delivery to a small surface area and its rapid turnover. The eye drop volume is only up to 50 µL[1]. In addition, the tear turnover may increase up to 5 times responding to the drop instillation, reducing the half-life of a drug from 4 min to 40 sec[2]. To circumvent these drawbacks, repeated administrations and high drug concentrations are often required to reach the desired therapeutic effects. This can be annoying, inconvenient, and expensive. It is also known that children, elderly, and disabled patients have problems in self-administering eye drops. To circumvent these drawbacks, bacterial cellulose could be used as drug carrier for corneal pathologies[3].

In this study, bacterial cellulose films were obtained by cultivating *Komagataeibacter xylinus* strain (NCIMB 5346, from CECT, Spain) in Hestrin−Schramm medium for 6 days. After cleaning with sodium hydroxide solution, bacterial cellulose was impregnated with drugs habitually used for the treatment of corneal pathologies (e.g., ciprofloxacin, ceftazidime, hyaluronic acid). Then, loading efficiency and pharmacokinetics studies were carried out. Molecular dynamics simulations were used to compute the surface adhesion energy between cellulose fibrils and antibiotics. The results will be discussed in light of the experimental release data.

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P5: The combination of cannabidiol and bacterial nanocellulose as wound treatment strategy

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Abstract

Cannabidiol (CBD), a natural drug from *Cannabis sativa*, is a promising compound that is currently investigated for many dermal treatment options. Recently, CBD has been shown to have an antimicrobial effect against different microorganisms, especially gram-positive bacteria [1]. As there is an unmet medical need to find new strategies for antibacterial wound treatment due to increasing bacteria resistance, in the present study a strategy for the dermal application of CBD in BNC was investigated.
BNC was used as delivery system because it has already demonstrated that its unique fibre network manifoldly supports the wound healing process [2]. However, BNC is highly hydrophilic, which hampers the incorporation of lipophilic components and an appropriate loading technique and loading medium had to be established [3].
The synthesis of the BNC fleeces was performed by cultivation of *Komagataeibacter xylinus*. To accomplish the incorporation of the lipophilic CBD into the hydrophilic BNC, the fleeces were loaded with a solution of 5 mg/mL CBD in propylene glycol using a submerse adsorption technique. The loading did not lead to any morphological change of the fleeces. A stable system was obtained without changes of the pressure stability compared to native fleeces. In addition, the loaded fleeces were more transparent, which improves the possibility of inspecting the wound ground.
To examine the antimicrobial activity, different concentrations of CBD in propylene glycol were tested against *Staphylococcus aureus* in an agar diffusion test. Even a concentration of 0.025 mg/mL showed an antibacterial activity. This antibacterial activity was maintained after the incorporation of the CBD in BNC. In conclusion, the combination of BNC with cannabidiol has been effective against *Staphylococcus aureus* and offers a possibility for the treatment of infections.

Acknowledgements

We would like to thank the KannaSwiss AG, Kölliken, Switzerland for providing cannabidiol and A. Fischer for her technical assistance.

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P6: Highly porous and stretchable novel bacterial cellulose membrane synthesized by *Komagataeibacter hansenii* SI

Izabela Cielecka, Małgorzata Ryngajłło, Maniukiewicz Waldemar, Stanisław Bielecki

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Abstract

Bacterial nanocellulose (BNC) as a biomaterial is well known for its unique properties, especially its high tensile strength, porosity, and hierarchical structure. Most *Komagataeibacter* strains produce cellulose with a dense, three-dimensional fiber structure that is resistant to tensile force. The porosity of this biopolymer is one of the key properties in diverse applications. BNC synthesized under static conditions exhibit high porosity, but the pore size is insufficient for use in tissue engineering, *ex situ* modification, or as a matrix for drug and enzyme delivery. To date, the properties of BNC have been mainly controlled by chemical and physical modifications of the final fermentation product [1,2] or by genetic modifications of BNC producers [3]. Only to a limited extent, the possibility of strongly influencing the properties of BNC in situ, by changing the medium compositions and culture conditions to newly isolated strains from nature, has been investigated.

In the present study, a new cellulose-producing strain was isolated and identified as *Komagataeibacter hansenii*, named SI1 [4]. The bacterial cellulose synthesized by this strain exhibits remarkable tensile properties such as the ability to stretch up to 70-120%. It can be manually stretched in any direction without destroying the fibers, and it retains its shape after elongation. SEM (scanning electron microscope) observations showed that the cellulose membrane structure is highly porous with large spaces between the fibers. We will present some aspects of stretchable bacterial nanocellulose (SBNC) biosynthesis, from strain characterization and its kinetics of growth to the study of culture conditions for improved stretching ability, to the characterization of key properties of the novel membrane of SBNC.

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P7: Bacterial cellulose materials: Our recent topics and future concepts

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Abstract

The biotechnological company "KKF – polymers for life" was founded in 2005 by a spin-off of scientists from Jena University. The development of novel and innovative products in the field of synthetic and natural polymers – especially bacterial cellulose or so-called biocellulose (BC) – together with partners from industry, universities, clinics and scientific institutions is in the focus of our recent topics and future concepts introduced in the poster presentation.

An ongoing highlight of KKF R&D is still directed to organ regeneration. The current state of BC implant design will be presented. Close to that, the development of multilayered BC-gels for medical use are a further topic. These gels are created biotechnologically and via 3D-bioprinting. As an exciting feature of BC is their shaping power. At the poster selected examples of flat and tubular composites will be shown.

A further important business segment contains challenging tasks concerning BC surface modifications as well as coating technologies. One topic is the question: Can “Chemization” of shaped BC implants be a promising way to improved surfaces? A further one deals with two important ways for BC coatings: biotechnological deposition and BC liquid applying. As a novel idea we investigate the way from BC nanofiber network to crosslinked surface protecting layer. Finally, laser-perforated BC coatings and membranes are also in the focus of our work.

Acknowledgments

The authors would like to thank Dr. Ulrike Udhardt, Dr. Friederike Kramer, and Thomas Richter for their long-term, creative, and comprehensive activities for the development of bacterial cellulose in Jena.

P8: Cellulose - A potential biopolymer from micromachineries

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Abstract

The demand for cellulose is increasing rapidly owing to its desirable properties such as biodegradability, affordability, availability, recyclability and ecofriendly. This demand will continue to scale up in the forthcoming years as cellulose has the potential to replace petroleum-based products for sustainability, and cost effectiveness. Bacterial Cellulose (BC) is an alternative pathway leading to paradigm shift from the conventional practices of extraction and purification of celluloses or nanocelluloses from plant fibre to micromachinery-based BC, sparing the forests and the land from massive overexploitation. BC is finding applications in several industries such as biomedical, food, cosmetics, packaging industries, food additives etc. for its purity and biocompatible nature. For production of BC, media optimization is the primary key to know the effective concentration for the highest production of cellulose from bacteria. In the present study, *Leifsonia soli* was exploited to produce BC and CCD based RSM statistical optimization method was adapted for culture parameters optimization. The combination of optimum condition of pH 6.5, temperature 37^{O} C, calcium chloride as inducer, 10 % inoculum concentration in presence of maltose as carbon source and soy whey as nitrogen source gave the highest yield of bacterial cellulose (7.5 g/l). The obtained BC can find its application in medical sectors particularly in scaffold preparation and drug delivery.

P9: Preparation of bacterial cellulose/polyvinylidene fluoride with micro-nano fibrous structure for small-diameter vascular grafts

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Abstract

Artificial blood vessel grafts have already widely used in clinical treatment for cardiovascular disease [1, 2]. However, small-diameter vascular grafts have always been the focus and difficulty of research as rapid endothelialization is hard to achieve after implantation, leading to complications such as thrombosis and intimal hyperplasia. Considering that the natural vascular intima is composed of nano- to micro-scaled fibers, the construction of small blood vessels with a biomimetic micro-nano fibrous structure is expected to provide a new opportunity for rapid endothelialization. In this work, polyvinylidene fluoride (PVDF) microfiber scaffolds were prepared by electrospinning, which was coated with polydopamine to make hydrophilic surface. Then, bacterial cellulose (BC) nanofibers were grown in the pores of PVDF scaffolds by static culture method to form a small-diameter blood vessel with micro-nano fibrous structure. As seen in Fig. 1a-e, scanning electron microscope (SEM) characterization showed that BC nanofibers (diameter: 45 ± 1 nm) and the PVDF microfibers (diameter: 694 ± 17 nm) formed an entangled three-dimensional micro-nano bionic network structure, which was similar to the structure of the natural vascular intima. The tensile strength (3.47 MPa) of BC/PVDF was superior to that of BC (0.81 MPa) and PVDF (2.89 MPa) (Fig. 1f), and higher than that of thoracic aorta (1.95 MPa). Moreover, the small-diameter blood vessels had good biological properties. Endothelial cells extensively spread on the surface of the BC/PVDF graft after seeding for 5 days, suggesting that rapid endothelialization could be achieved. In summary, the BC/PVDF graft with a biomimetic micro-nano fibrous structure is expected to be a candidate for the small-diameter vascular graft with rapid endothelialization function.

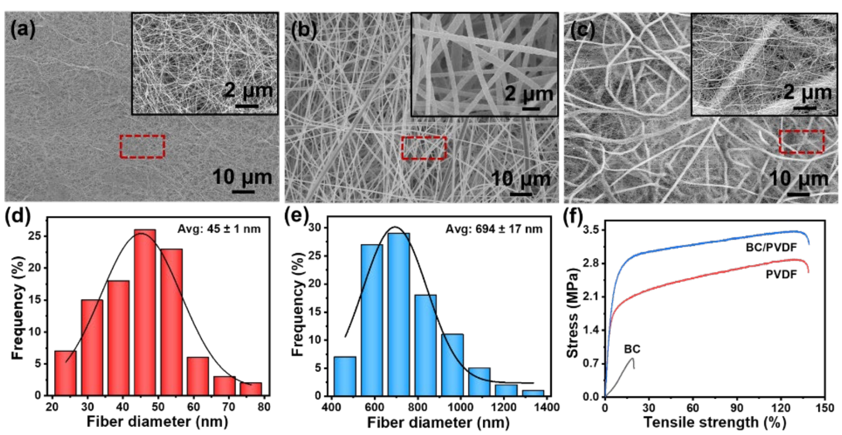

Figure 1. SEM images of BC (a), PVDF (b) and BC/PVDF (c); Fiber diameter distribution of BC (d) and PVDF (e); Stress-strain curves of BC, PVDF and BC/PVDF (f).

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P10: Construction of polydopamine/bacterial cellulose film for vascular graft application

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Abstract

Bacterial cellulose has received great attention as vascular graft due to its unique structure and properties, such as 3D network structure similar to natural extracellular matrix, appropriate mechanical properties, and excellent biocompatibility. However, it is documented that the mechanical properties and biocompatibility of BC should be further improved [1, 2]. Herein, we designed a polydopamine (PDA)-modified bacterial cellulose (BC) film by in-situ method. Scanning electron microscopy observation showed that the PDA/BC film retained the inherent 3D network structure of BC. Mechanical property test results (Fig. 1a and b) showed that compared with the pure BC film, the tensile strength of the PDA/BC film increased by about 77% (0.9 MPa for BC film), and the Young's modulus increased by about 154% (3.5 MPa for BC film), indicating that the PDA/BC film exhibited improved mechanical properties over the pure BC film. Furthermore, more endothelial cells were observed on the surface of the PDA/BC film than those of the pure BC film (Fig. 1c and d). The CCK-8 experiment showed that the PDA/BC film was non-toxic, indicating that the PDA/BC film had favourable biocompatibility. In summary, the PDA/BC film might be a promising candidate material as vascular graft due to its excellent mechanical properties and biocompatibility.

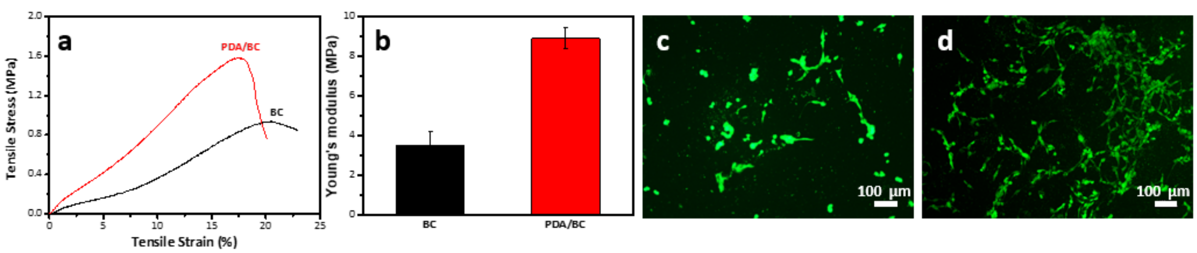

Figure 1. Stress-strain curves (a) and Young's modulus (b) of BC and PDA/BC; FDA staining images of endothelial cells co-cultured with BC (c) and PDA/BC (d) films on day 5.

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P11: Preparation and properties of micro/nano-fibrous structred vascular grafts

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Abstract

Artificial vascular grafts have been extensively explored. Due to the special physiological environment of small-caliber blood vessels (< 6 mm), it is a challenge to prepare small-caliber vascular grafts with rapid endothelialization. The intimal basement membrane to which endothelial cells are attached is similar in fiber composition to the micro/nano-fibrous combination, which plays a key role in endothelial cell adhesion, migration, proliferation, and differentiation. Mimicking the native vascular intimal structure is considered to be an effective strategy to construct vascular grafts with rapid endothelialization. Herein, bacterial cellulose (BC) nanofibers were combined with electrospun polyethersulfone (PES) microfibers to construct a vascular graft imitating the intimal basement membrane with a micro/nano-fibrous structure. The present study constructed PES microfibers tubes by electrostatic spinning. Subsequently, threaded BC nanofibers into PES microfibers by in situ culture to obtain a vascular graft with micro/nano-fibrous structure. It had micro/nano-fibrous morphology and well-interconnected microchannel structure, which provided a good microenvironment for cell adhesion and proliferation. Fluorescent staining demonstrated that cells cultured on the BC/PES spread and proliferated well. This study provides a possible guidance for the preparation of vascular grafts.

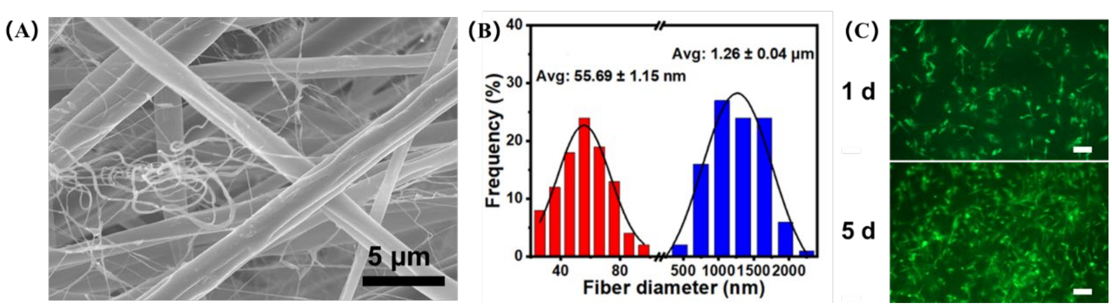

Figure 1. (A) SEM image of BC/PES composites; (B) Fiber diameter distribution of BC and PES in BC/PES composites; (C) Images of the fluorescent staining of fluorescein diacetate of HUVEC seeded on the BC/PES grafts after cultivation for 1 and 5 days. Scale bar: 100 μm.

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P12: Influence of fermentation time on the mechanical properties of bacterial cellulose

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Abstract

Cellulose is the most abundant natural polymer on the planet. Although plants are the main sources of cellulose, some bacteria can synthesize this polymer, originating the bacterial cellulose (BC). BC has a chemical composition like vegetable cellulose (VC); however, this nanostructured polymer stands out for its chemical and physical properties, usually superior to those of VC [1]. These properties guarantee BC a wide range of applications, such as acoustic membranes, food packaging, wound dressing, etc. Depending on the application, different properties are desirable for the product to have the highest quality [2,3]. These properties can be modified chemically, biologically, physically, or by changes in cultivation parameters during fermentation. The influence of fermentation time on the mechanical properties of BC was studied. Different fermentation times (4, 6, 8, and 10 days) were evaluated in static culture (30 °C) by the bacterium *Komagataeibacter xylinus* ATCC 53582 in HS synthetic culture medium. The BC pellicles were purified and dried for further mechanical testing and analysis of the degree of polymerization (DP). The DP values obtained are within the expected standard for BC (2000 - 6000), which represents a high DP when compared to that of vegetable cellulose (300 - 1700) and increased with the increase of the time of fermentation, with values of ~2931 (BC 4 days), ~3373 (DC 6 days), ~3398 (BC 8 days), and ~3719 (BC 10 days). The BC samples showed increasing values of maximum stress, stress at rupture, and Young's modulus with increasing fermentation time used, going from 133 to 254 MPa for maximum stress, 135 to 255 MPa for stress at rupture and 6,4 to 11 GPa for Young's modulus. The sample with the best results of mechanical properties and degree of polymerization was BC 10 days, indicating that mechanical properties increased with the increase of the DP as well as the fermentation time, showing a directly proportional correlation between these two properties. This high mechanical strength aligned with a high degree of polymerization can direct the application of this sample to the food industry either in the packaging sector or as a food thickening agent since high degree of polymerization and great mechanical properties are of fundamental importance for these types of applications. It is concluded that it is possible to change the properties of the CB during the fermentation process and, according to the difference between the properties, the applications for the CB can be defined.

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P13: Antimicrobial activity of bacterial nanocellulose modified with chestnut extract

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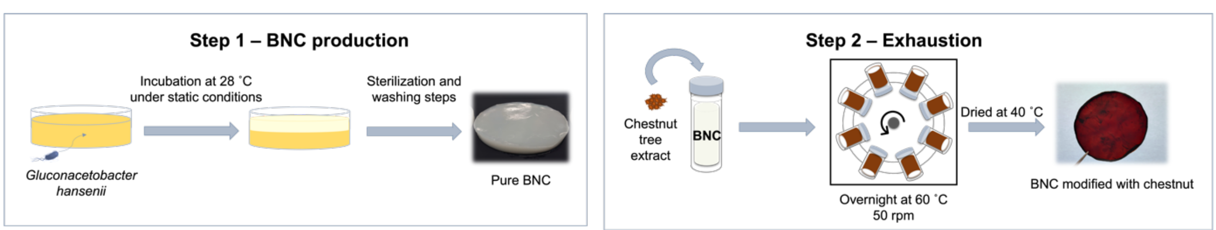
Abstract

Chestnut wood and bark extracts are rich in tannins. Hydrolysable tannins exhibit numerous health-promoting properties such as antioxidant, antimicrobial, anti-parasitic, anti-inflammatory, anti-carcinogenic, anti-ulcerative, antiangiogenic, phytoestrogenic, and P-glycoprotein inhibiting effects [1, 2]. The most abundant polyphenolic compounds in chestnut extract are hydrolysable tannins (gallotannins and ellagitannins), where vescalagin and castalagin are the most important constituents (nearly 10 %), contributing significantly to the chestnut's antimicrobial activity [2]. Therefore, chestnut extract has tremendous potential to be used in medical appliances. The incorporation of chestnut extract within the nanofibrous structure of bacterial nanocellulose (BNC) produced by *Gluconacetobacter hansenii* ATCC 53582 was obtained through exhaustion. The chestnut extract adsorbed tightly onto the surface of the nanofibers and across the entire depth of the membranes, resulting in functionalized BNC with similar properties to those of the chestnut extract. However, BNC became more brittle. Adding glycerol as a plasticizer circumvented this issue, resulting in a highly flexible and resistant material. The antimicrobial activity of the chestnut modified BNC was tested against common bacteria: *Escherichia coli* and *Staphylococcus aureus*, MS2 bacteriophage, and yeast *Candida parapsilosis*. Antioxidant properties, release profile and swelling behavior were evaluated. Morphology of the functionalized BNC was analyzed through scanning electron microscopy, and the chemical composition using Fourier transform infrared spectroscopy. In this study, the simple processing methodology resulted in a flexible, biodegradable, biocompatible nanocomposite for potential application in medical appliances, including skin injuries in particular for diabetes wounds.



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P14: The microscale morphology of bacterial cellulose produced by *Gluconacetobacter hansenii*: anisotropy and liquid-crystalline ordering

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Abstract

The microscale morphology of bacterial cellulose (BC) strongly depends on the cultivation conditions as well of the producer strain used. BC mass can possess an anisotropic layered organization, if cultivated under static conditions. The layers of interwoven fibrils are oriented parallel to the air-liquid interface. The morphology has an essential impact on the mechanical, water absorption, diffusion rate properties of the BC mass.

Varying different parameters of the *Gluconacetobacter hansenii GH-1/2008* cultivation protocol, we have found a strong evidence that the microscale morphology is dominated by the BC production rate, in the first place. We revealed that BC films obtained under static cultivation conditions, at higher production rates, demonstrated liquid-crystalline-like left-handed helical alignment. Meanwhile, in BC mats grown at lower rates, no liquid-crystalline ordering was detected. For BC pellets (spheroids) grown by agitation method, the morphology also depended on the conditions used. Particularly, the agitation rate, and, therefore, the oxygen availability influenced not only the shape and size of the particles, but their inner structural organization.

In this report, we analyze the origin of the layered structures as well as factors and putative mechanisms that give rise to them.

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P15: Production of Bacterial Cellulose-Based Shape Memory Bone Tissue Engineering Scaffolds

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Abstract

Bone scaffolds produced from shape memory polymers, a class of smart materials, have a significant potential for irregular shaped bone injuries [1], [2]. Bacterial cellulose (BC) with properties such as high water holding capacity, high purity, high crystallinity, high mechanical strength and ease of modification is used to obtain porous tissue scaffolds by biomimetic modification with hydroxyapatite (HAp) to produce bone tissue engineering scaffolds [3], [4]. In this study, BC membranes produced under static culture conditions were fragmented with the aid of a homogenizer after purification. Fragmented BC fibers were treated with $CaCl_2$ for three days, and with 1.5xSBF (simulated body fluid) for seven days for biomimetic HAp precipitation, and at the end of the period, the samples were poured into moulds and lyophilized to obtain bone tissue scaffolds. The use of fragmented BC enabled biomimetic HAp synthesis to be homogeneous throughout the structure and made BC pore diameters, which are very low under normal conditions, suitable for bone tissue engineering. In SEM images of BC-HAp scaffolds, it was observed that biomimetic HAp crystals were homogeneously dispersed within the structure and the pore distribution was concentrated in the range of 160-235 µm. In addition, the water holding capacity of BC-HAp scaffolds was determined to be approximately 60 times their dry weight. BC-HAp scaffolds were exposed to various shape deformations and were found to be restored when in contact with liquid. The obtained shape memory porous BC-HAp scaffolds are expected to swell when they come into contact with body fluids in the area of irregular bone tissue damage, allowing the damaged area to be filled completely. BC scaffolds promoted cell differentiation. As a result, a BC based tissue scaffold with suitable pore diameter for bone tissue engineering and suitable for irregular craniomaxillofacial bone injuries has been produced.

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P16: Micro Computed Tomography (μ-CT) Evaluation of Hydroxyapatite Functionalized Bacterial Cellulose Scaffolds for Bone Tissue Engineering

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Abstract

Bacterial cellulose (BC) is used as a scaffold material in many different tissue engineering products since it successfully mimics the fiber structure of the extracellular matrix proteins [1-4]. This study aims to analyse the porosity and pore distribution of hydroxyapatite (HAp)-functionalized and unfunctionalized BC by Micro Computed Tomography (CT) and to determine the suitability of these scaffold for bone tissue engineering applications. There are several assessment techniques of porosity; however, Micro-CT is one of the advantageous instruments for this purpose. It is a non-destructive imaging technique and it can quantify the pore network in a three-dimensional way. BC membranes produced under static culture conditions were fragmented by homogenizer after purification. Fragmented BC was treated with $CaCl_2$ for three days, and 1.5x simulated body fluid for seven days for biomimetic HAp precipitation. At the end of the period, the samples were placed in moulds and lyophilized to obtain bone tissue scaffolds. The samples were placed into sample holders and scanned at 70 kVp energy, 114 μA intensity, 300 ms integration time, and 10 μm voxel size parameters in the Micro-CT device, and 3D analyses were performed with an evaluation program. The mean pore diameter and porosity of BC-HAp composites were 125.5±36.8 μm and 92.3%, while the mean pore diameter and porosity of BC were 143.8±48.1 μm and 92.4%, respectively. As expected, a decrease in the pore diameters of BC-HAp was observed, due to the presence of HAp particles deposited on the BC fibers. However, no significant difference was observed in porosity between the BC and BC-HAp scaffolds. As a result of the study, it was determined that the required porosity and pore diameters for cell migration and mass transfer were obtained, and it was seen that the scaffolds could be used in bone tissue engineering applications.

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P17: Effects of Chitosan on Chondroitin Sulfate Binding to Bacterial Cellulose-Based Tissue Scaffold

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Abstract

Cartilage tissue engineering aims to treat cartilage damage by seeding chondrocytes or stem cells that differentiate into chondrocytes, and applying them to the damaged area using tissue scaffolds [1]. The scaffolds used should be biocompatible, have high mechanical strength and porosity, and mimic the cartilage microenvironment. Bacterial cellulose (BC) has high biocompatibility, water holding capacity and gas permeability, as well as suitable fiber structure and mechanical properties for utilization as a cartilage tissue engineering scaffold. With their glycosaminoglycan-like structures, chitosan and chondroitin sulfate stimulate proteoglycan and type II collagen secretion in articular cartilage [2]–[5].
In this study, it is aimed to provide a suitable microenvironment for bone marrow mesenchymal stem cells to differentiate toward chondrocytes by mimicking the cartilage extracellular matrix with the modification of BC, chitosan and chondroitin sulfate. BC-Chitosan$_{0.1}$, BC-Chitosan$_{0.25}$, BC-Chitosan$_{0.5}$ scaffolds using different amounts of chitosan were produced using fragmented BC and the obtained scaffolds were lyophilized. The effects of chitosan on chondroitin sulfate absorption onto BC surface were determined by DMMB assay. It was observed that the chitosan modification at different concentrations did not make a significant difference in terms of cell viability, but the chondroitin sulfate holding capacity of the BC-Chitosan scaffold with the highest chitosan concentration was higher. In the BC-chondroitin sulfate scaffolds, absorbed percentage of chondroitin sulfate was 0.98%, while the percentage of chondroitin sulfate absorption increased to 44% as the chitosan concentration increased.

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P18: Pore Optimization in Bacterial Cellulose Membranes with Polycaprolactone Microspheres and Demonstrating the Migration of Monocytes through the Porous Membrane

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Abstract

Basal laminae are flexible and 40-120 nm thick special extracellular matrix mats that separate epithelial, endothelial, muscle, fat and nerve cells from the surrounding connective tissues. Besides their structural and filtering roles, they influence cell polarity, cell metabolism, cell survival, proliferation, or differentiation, and are involved in cell migration [1-3]. Cell migration analysis plays an important role in studying physiological processes, such as host defence, wound healing, cancer metastasis and embryogenesis. In physiological and pathological conditions, mononuclear phagocytes are the first cells to migrate to tissues and infiltrate from the basal lamina [4, 5]. Modelling the basal lamina between the two cell layers is critical for the investigation of cell migration *in vitro*. Porous membranes and hydrogels are used to model the migration of mononuclear phagocytes, such as neutrophils and monocytes and inflammatory cells to wounds and other lesions.

Bacterial cellulose (BC) is a biopolymer with unique mechanical/structural properties and high purity, with its nano-porous structure. Recently, we have used BC as a physical barrier that restricts cell migration and allows the metabolites to pass through in an *in vitro* blood-brain-barrier model with various modifications [6]. In this study, BC was modified using polycaprolactone microspheres, and pore size expansion was achieved to allow monocyte migration analysis. Produced BCs were shown to have a pore diameter of 4-10 µm using the ImageJ program on the images taken by scanning electron microscopy. While monocyte cell migration was not observed in unmodified BC membranes, fluorescent markers showed that monocytes migrated through the porous BC membrane in the presence of apoptotic cells. A membrane that can be utilized in various *in vitro* migration studies has been developed by demonstrating the migration of monocytes through porous bacterial cellulose.

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P19: Production of Bacterial Cellulose Using Food Processing Wastes for Biomedical Applications

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Abstract

Cellulose is one of the largest renewable and biodegradable biopolymer found in nature. Cellulose is commonly obtained from plants, but it can also be sustainably synthesized by aerobic bacterial species including Acetobacter.1 Bacterial cellulose (BC) and its derivatives have a huge market demand due to their tremendous commercial applications in multiple areas including the biomedical and food industries. However, the production cost of BC using the standard Hestrin-Schramm(H-S) media is quite expensive, especially at a commercial scale. Fortunately, the advances in biotechnological techniques have led to the production of BC using renewable industrial wastes from the food and agriculture industries. This waste utilisation strategy makes BC a cost-effective and renewable raw material, making it also beneficial in the management of wastes and in the reduction of the reliance on plant cellulose.2 The primary focus of this research is to produce BC cost-effectively using reformulated media from food processing wastes to develop commercially viable source of hypoallergenic biopolymer for various biomedical applications. So far in this study, a cellulose-producing bacterium has been isolated from apple cider vinegar. The isolate is a Gram-negative, aerobic, rod-shaped bacteria, with optimal BC temperature of 28°C. Its biochemical characterisation reveals that it belongs to the acetic acid bacteria group. Sequencing of 16SrRNA gene and subsequent phylogenetic analyses reveals that it is 99% similar to Gluconacetobacter entanii. The Gluconacetobacter genus is the highest producer of BC. The growth of this isolate is better in agitated culture method whereas the production of BC is higher in static culture method with an optimal pH of 6. Physical, spectroscopic, and biochemical characterisation of the biopolymer confirms that the secreted exopolysaccharide is cellulose. Food processing wastes with high protein and carbohydrate content such as rice bran and cereal dust were successfully used to substitute the carbon and nitrogen sources of the standard H-S media. Different sets of modified waste media gave 80% similar yield compared to the H-S media, which demonstrates that rice bran and cereal dust are suitable bacteriological media alternatives to produce BC that is 95% cheaper. Lastly, the BC produced from waste media will be tested and functionalised for cell adhesion and proliferation to develop tissue scaffolds for tissue engineering applications.

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