LULEÅ UNIVERSITY OF TECHNOLOGY

TANDEM FOREST VALUES

14 th August 2023

Prof. Kristiina Oksman
E-mail: kristiina.oksman@ltu.se
Mobile 070-3585371
Phone office 0920-493371

Project report: Hierarchical 3D-structured nanocellulose aerogels and networks for use in biomedical applications

Summary project outcomes

The objective of the collaboration project was to develop functional and hierarchical 3D-nanostructured membranes derived from forest biomass for use as ultrafiltration membranes or adsorbents and broaden our fundamental knowledge on nanocellulose and its surface modifications for separation and isolation of biological exosomes, viruses, or bacteria in medical uses. The originality and innovativeness of the conducted work is very good as we have used processes which have not been used before for development of membranes or adsorbents for exosomes (EXs). These topics are expected to provide breakthrough openings in biomedical diagnostics. The 3D structures are also expected to be useful for capturing bacteria and viruses depending on their pore size and form. As well as low-cost wood nanomaterials have enormous possibilities for future medical uses and the use of cellulose would take forest biomaterials to new high-tech applications. Our aim is to continue the collaboration related to developed materials and we are planning to apply funding for the development of the applications. In addition, we already obtained a new project funding in which forest-based materials are developed for sustainable electronics in collaboration with LTU and University of Oulu.

Persons involved in the project at Luleå University of Technology (LTU) Sweden and at University of Oulu (UO) Finland:

Professor Kristiina Oksman, Wood and Bionanocomposites, LTU, kristiina.oksman@ltu.se
Dr Rasoul Esmaely, Wood and Bionanocomposites, LTU
Dr Reny Thankam Thomas, Wood and Bionanocomposites, LTU
MSc. Jose Ignacia del Rio De Vicente, Wood and Bionanocomposites, LTU
Professor Henrikki Liimatainen, Fibre and Particle Engineering, UO
Dr Kaitao Zhang, Fibre and Particle Engineering, UO
Dr Mohammad Karzarjeddib, Fibre and Particle Engineering, UO
Professor Seppo Vainio, Biocentre, UO
Dr. Feby Pratiwi, Biocentre, UO

The project had different strategies to develop porous membranes with tuned functionality and it was divided in three tasks: *1) Functionalization of nanocellulose, 2) Materials processing and 3) Testing.* Task 1 was led by the OU, Task 2 by LTU and in Task 3 all partners were involved. Electrospinning of membranes with polyvinyl acetate and cellulose acetate polymers were developed and different type of nanocelluloses were used to create novel functionalities. In addition, we tested cellulose hydrogels which were freeze dried to 3D-cellulose aerogels and coated with functionalized cellulose nanofibers. These structures were formed to have hierarchical pore structure with nano- and micro-size pores to be used as filtration membranes or adsorbents.

Task 1 Functionalized nanocellulose (UO)

A new derivatization approaches was used to obtain chemically functionalized and bioactive substances containing cellulose nanocrystals and cellulose nanofibers that have an affinity towards biological entities. Especially, functionalization with cationic groups and antibodies using deep eutectic solvents was conducted which led to positively charged nanocelluloses. Reference nanomaterials were bacterial cellulose, which are pure cellulose nanofibers without any surface charge and anionic cellulose nanocrystals, which are negatively charged.

Task 2 Materials processing (LTU)

Hierarchical 3-dimensional porous membranes were prepared using electrospinning and lyophilization of cellulose hydrogels. These methods led in very interesting nanostructured materials with high porosity and they were tested as filtration membranes. The electrospinning was made with Fluidnatek LE-10. In this process the network structure is depending on spinning precursor properties as well as needle type, working voltage, spinning speed and type of collector. The challenge in electrospinning processes was the choice of the precursor polymer. We started with polyvinyl acetate (PVA) and faced many problems, and this precursor was then changed to cellulose acetate (CA). The solid content of CA was tested, and it was found that 12 wt% led to the best structure, shown in Fig. 1.

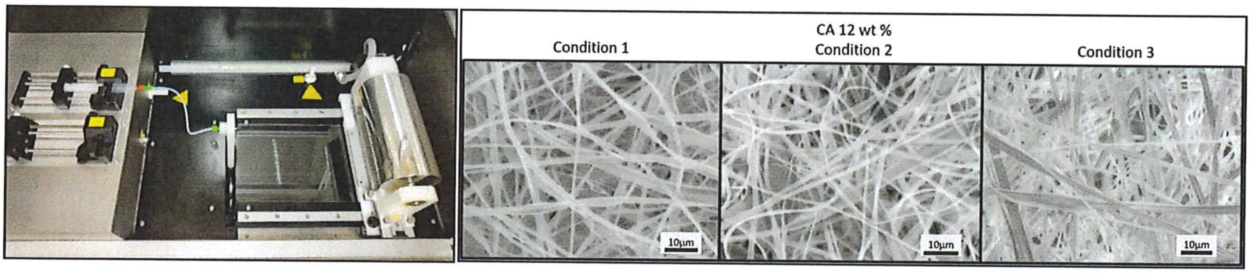

Fig.1: Electrospinning equipment and example of optimization, CA 12% solid content with three different spinning conditions.

Instead of 3D printing cellulose hydrogels, we started with bacterial cellulose (BC) which is a natural hydrogel with hierarchical 3D structure similar with what was planned originally to be produced with 3D bioprinting. The hydrogels were freeze-dried into aerogels and coated with cellulose nanofibers using a similar filtration method as with the electrospun membranes, see Fig. 2. But even though we showed that this could be a possible approach to produce membrane materials, we decided to focus on the electrospun membranes.

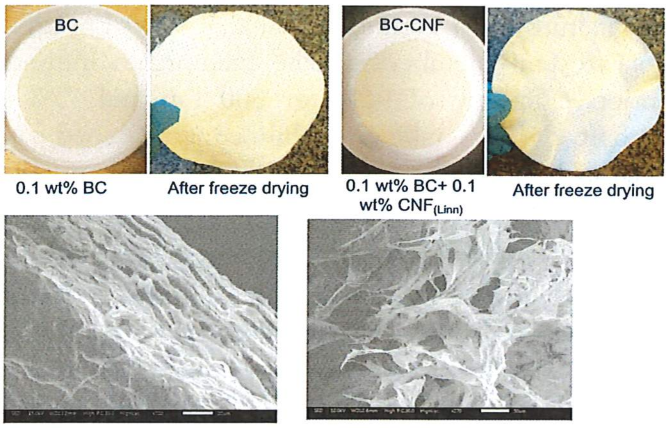

Fig.2: Porous nanofiber aerogel membrane (BC) before and after freeze drying and functionalized cellulose nanofiber coated BC membrane. Electron microscopy images of the membrane cross section and its porous structure.

Task 3 Testing of membranes and adsorbents (LTU and UO)

In this task the effect of surface functionalization of porous electrospun cellulose structures, and their charge, porosity and affinity towards different molecules and nanoparticles including biological exosomes were studied using a filtration setup. In addition, the mechanical properties, specific surface area, density and microstructures of membranes were studied using different technologies. Below is an example how image analysis was used to evaluate of the porosity of the functionalized electrospun membranes (Fig 3).

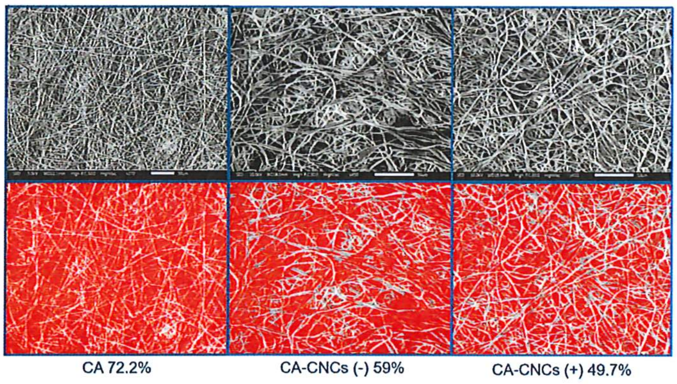

Fig.3: Evaluation of porosity by image analysis, contrasting the space between fibers for CA, CA- CNCs (-) and CA-CNCs(+).

The electrospun nanofiber membranes were first used for capturing different nanosized particles (MSc thesis and paper 1) and after that to biological exosomes (paper 2). Filtration capacity of the membranes produced was evaluated using fluorescence spectroscopy.

Main results

The objective of the project was to develop functional and hierarchical 3D nanocellulose structures for use as ultrafiltration membranes or adsorbents and to broaden our fundamental knowledge of nanocellulose and its surface modifications for separation and isolation of biological exosomes, viruses, or bacteria in medical uses. The project involved different strategies to develop porous membranes with tuned functionality.

Micrometer thick multifunctional and sustainable composite membranes of electrospun cellulose acetate (CA) infused with functionalized, anionic, and cationic cellulose nanocrystals (CNCs) with enhanced wettability, tensile strength, and excellent retention capacities were designed. CNCs could uniformly impregnate into the three-dimensional CA network to effectively improve its properties. The impregnation of cationic CNCs at 0.5 wt% concentration drastically increased the tensile

Division of Materials Science
Luleå University of Technology
SE-97187 Luleå, Sweden
PHONE: +46 920 49 33 71
WEBSITE: www.ltu.se

strength (1669%) while maintaining high permeation flux of 9400 Lm-2h-1 which is remarkable with cellulose modified electrospun membranes. The membranes infused with anionic CNCs exhibited a particle retention efficiency of 96% for 500 nm and 77% for 100 nm polymer nanoparticles whilst the cationic CNC membranes exhibited a combined particle retention strategy using selectivity and size exclusion with a retention of >81% with 100 nm polymer nanoparticles and 80% with ~50 nm silver nanoparticles.

The optimized all-cellulose electrospun membranes were sent to Biocentre, University of Oulu for further testing their potential for biomedical applications and these have shown to have unique performance. The membranes were shown to be suitable for collecting biological nanoparticles and the scientific publication is under preparation as well as a disclosure of invention. An example of the results when these were tested to capture biological nanovesicles and their retention (NVs) is shown in the Fig 4 below. Similar with the synthetic nanoparticles the retention of exosomes using functionalized membranes was excellent compared to the neat cellulose membrane.

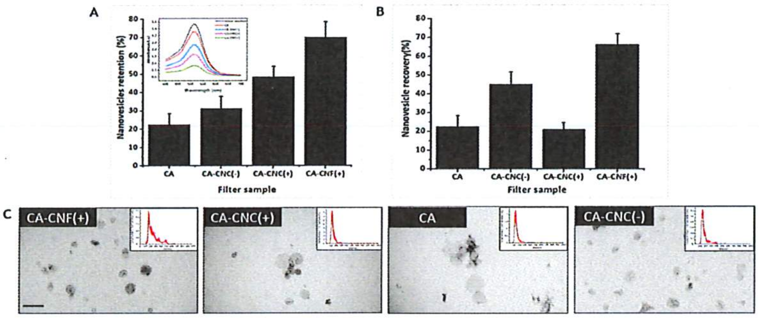

Fig.4: (A) The retention efficiency of all-cellulose membranes in capturing the NVs (CA = cellulose acetate, CNF (-) = anionic CNF, CNF(+) = cationic CNF), (b) the recovery efficiency of NVs eluted from the CNF, and morphology and size distribution of eluted NV from CNF

Our envision is that the developed multifunctional membranes can be utilized for affinity-based and size-exclusion filtration to selectively trap bacteria or substances of biological significance. CNCs with the criteria aiming at size exclusion separation and affinity-based filtration of nano-sized particles or a combination of both (Fig. 5).

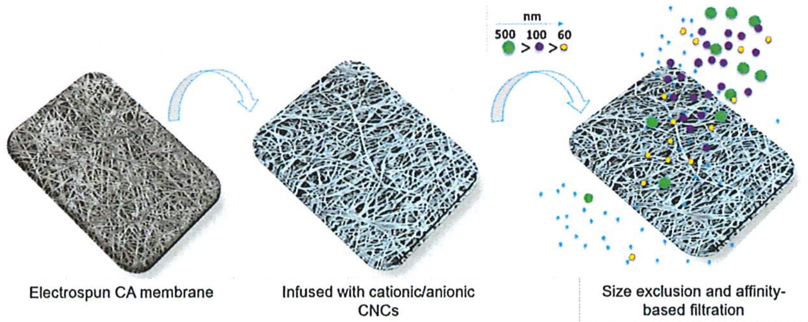

Fig.5: Functionalized all-cellulose electrospun membrane

The results of the project have led to a highly novel way of separation of biological nanosized particles from fluidic filtration medium and these results are of importance for biomedical applications which was the target. The final findings and disclosure of the invention are still under process.

Problems and changes compared to the original plan.

The project started at spring 2019 by recruiting the postdoctoral fellow Rasoul Esmaely from Iran at LTU. He was expert on electrospinning, and he started to work on electrospinning of the membranes using PVA as carrier polymer and with a literature study. However, he went back to Iran after few months because of a family issue and he didn't come back. After that a new recruiting process started and Dr Reny Thomas from India was recruited in the project at spring 2020. The visa process as well as the travelling from India to Sweden were difficult because of the corona restrictions and therefore her start was delayed to the October 2020. Additionally, the collaboration with Oulu University was affected because of the corona restrictions and it was not possible to travel between Sweden and Finland as planned. The collaboration between the Universities was done using online meeting and by sending materials between the universities.

Overall, the project was affected by the recruitment of people who did not stay the time according to the contract and by the pandemic which had a big impact on the project and restricted the travelling.

These are the main changes/reasons why the project has been behind the original time plan and why we are still working with the results on materials developed in the project. However, the scientific work has been successful and functional and hierarchical 3D-nanostructured membranes were developed from forest biomass with excellent behavior.

Conclusions

The electrospinning process of cellulose with tailored porosity and surface functionalized membranes for filtration of nanosized particles from filtration medium was developed in a collaboration project between Luleå University of Technology, Sweden, and University of Oulu in Finland. The overall results of the project were successful, and the collaboration has led to a novel method to separate nanosized particles by ultrafiltration and adsorption. Designed membranes infused with nanocellulose for size exclusion and affinity-based removal of nanoparticles (50 – 500 nm) and dye molecules. Membranes functionalized with cellulose nanocrystals enabled tunable porosity, surface functionality, improved tensile strength while maintaining high flux (9500 Lm-2h-1). Cationic nanocellulose functionalized membranes resulted in 80% retention of 100. These results are very promising and discussion about the further development in collaboration with the collaboration partners is ongoing.

The highlights of the results are:

- Cellulose impregnated electrospun membranes for multi-functional applications.
- Size-exclusion and affinity-based removal of nanoparticles is demonstrated.
- CNC enabled fabrication of membranes with tuned porosity and surface functionality.
- The cationic membranes showed excellent nanoparticle retention.

Effect of Tandem Forest Values Grant

The grant has contributed to competence building, which facilitates and strengthens the long-term collaboration between Luleå University of Technology in Sweden and Oulu University in Finland. The partners have an ongoing collaborative project within the regional EU project Interreg AURORA program and plan to seek new funds for the research on "separation of biological particles". The TANDEM FOREST VALUE project grant has also led to a new collaboration between LTU and the Biocentre at Oulu University.

The project has been interdisciplinary and combined expertise in materials science, chemistry, biomedicine, and nanotechnology and this has led to potential new materials and applications for biomedical and related fields. Despite pandemic problems and many restrictions, we have faced, the project results are very promising and have led to an innovation and strengthened the scientific edge and the new knowledge at LTU and UO. It has led to a master's thesis at LTU, and joint publications, new types of innovation and new research projects that can be collaborations with industrial partners and end users.

The results show that forest resources are excellent raw materials for functionalized membranes and can be based on affinity and size exclusion filtration and can also be used in biomedical applications. The forest industry is looking for new uses for the forest raw material and biomedical applications are a potential use, as they do not represent traditional bulk products but have a high value and are expected to be an important and emerging market area in the future. We believe that the developed membranes could be interesting for forest companies such as UPM or Stora Enso (Finland and Sweden) that have shown interest in medical applications. In Sweden, traditional medicine companies such as AstraZeneca may be interested. In addition, several start-up companies related to new biomedical products in the Oulu area have the potential to further develop the achieved results. The fact that nanocellulose can be used to functionalize the membranes to capture exosomes, viruses or bacteria would have specific technical and market advantages over many other materials because these cellulosic materials are not only bio-based but also non-toxic and compatible with the human body. Possible future collaborators have not yet contacted as the study is still ongoing as well as the invention process.

Best regards,

Kristiina Oksman
Chaired Professor

Margareta Groth
Head of the Department

Department of Engineering Science and Mathematics
Luleå University of Technology

References

1. Cellulose nanocrystal functionalized cellulose acetate electro spun membranes for adsorption and separation of nanosized particles, Jose Ignacia del Rio De Vicente, Master thesis, Luleå University of Technology, 2021.
2. Thomas RT, Del Río de Vicente JI, Zhang K, Karzarjeddi M, Liimatainen H, Oksman K. Size exclusion and affinity-based removal of nanoparticles with electrospun cellulose acetate membranes infused with functionalized cellulose nanocrystals, Materials & Design, 217 (2022), 110654. https://doi.org/10.1016/j.matdes.2022.110654
3. Pratiwi F, Thomas R, Karzarjeddib M, Liimatainen H, Vainio S, Oksman K. Efficient purification method of exozomes from natural sources using all-cellulose membranes (manuscript under preparation).

Appendix 1. Financial accounting

LTU Final Report Tandem Forest Values								
2018-2022								
Personnel		**2018-10**	**2019**	**2020**	**2021**	**2022**	**Total**	
Postdoc		0	452 126	189 125	660 568	229 718	1 531 536	
							0	
							0	
		0	**452 126**	**189 125**	**660 568**	**229 718**	**1 531 536**	
Other costs							0	
Premesis	15,20%	0	72 340	24 586	99 085	36 755	232 766	
IT-costs	3,40%	0	15 372	6 430	22 459	7 810	52 072	
Travel		0	12 881	3 165	0	8 572	24 618	
Consumables		0	14 150	5 799	3 281	5 710	28 941	
Analysis		0	0	0	6 036	1 600	7 636	
Publications Open access		0	0	0	0	29 445	29 445	
Other costs		0	42 404	15 395	31 776	53 138	142 712	
Total Other costs including premesis		0	114 744	39 981	130 861	89 892	375 479	
Indirect costs	37%	0	167 287	69 976	270 833	94 184	602 280	
Total Costs for the project		**0**	**734 157**	**299 081**	**1 062 262**	**413 794**	**2 509 295**	**100,00%**
Approved KSLA							**2 000 000**	**79,70%**
<u>LTU Co-funding</u>								
Premesis							**232 766**	
Publications Open Access							**29 445**	
LTU Co-funding in total							**262 211**	**10,45%**

Division of Materials Science
Luleå University of Technology
SE-97187 Luleå, Sweden
PHONE: +46 920 49 33 71
WEBSITE: www.ltu.se