**National Institute for Research & Development in Chemistry and Petrochemistry ICECHIM Bucharest**

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**Team 12 Polymer composites and nanocomposites**

**Nanocellulose 3D structures for regenerative medicine (CELL-3D)**

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**Project summary**

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Regenerative medicine and tissue engineering may offer new hope of life in case of loss or malfunction of a tissue or organs. One of the key elements in tissue engineering is the three-dimensional (3D) scaffold which provides structural support for cell attachment, proliferation and differentiation. Biopolymer scaffolds are preferred in tissue engineering for avoiding the issues related to donor immune rejection and pathogen transfer. Cellulose, the most abundant natural polymer on earth, is an almost inexhaustible source of valuable materials and has gained high interest in the form of nanocellulose (NC). NC stands out for its high crystallinity and mechanical strength, good biocompatibility, high water-holding capacity and large surface area and it has been intensively studied for medical applications because it does not cause toxic or allergic side effects in contact with living tissue. 3D scaffolds should possess a network of interconnected pores to permit cell migration, diffusion of nutrients and clearance of wastes, they must be able to support cell adhesion and promote cell growth and they must have balanced stiffness-toughness properties to support and transfer of loads. To meet all these requirements in porous 3D scaffolding, CELL-3D has as main objective to design new 3D nanocellulose structures that can be applied in regenerative medicine.

**Phase I - 2017**

In the first phase, porous nanocellulose structures have been obtained starting from several nanocellulose sources: (i) nanocellulose obtained from the mechanical disintegration of bacterial cellulose membranes, (ii) nanocellulose obtained by acid hydrolysis of microcrystalline cellulose, (iii) nanocellulose from agro-industrial waste (plum seeds shells), (iv) nanocellulose obtained by tempo oxidation and mechanical defibrillation and (v) nanocellulose obtained by defibrillation and plasma functionalization. Nanodimension of nanocellulose samples was highlighted by AFM, SEM and TEM. To obtain porous structures with controlled properties nanocellulose samples were functionalized by silanes and crosslinked using non-toxic crosslinking agents with different efficiency. In another approach, ionic agents have been used to obtain nanocellulose hydrogels and cryogels. These methods led to 3D structures with controlled pore sise and interconnected micro- and nano-pores. The porous 3D structures were characterized to observe the influence of the treatments on morphology, structure, thermal and mechanical properties and to detect the influence of the treatments on these characteristics. The methodology to obtain functionalized nanocellulose was elaborated. Moreover, the scientific report on the correlation between the process parameters, composition and the thermal and mechanical properties as well as morphology of 3D functionalized nanocellulose structures was released. High resolution computerized tomography revealed a porous structure with over 80% open porosity and a high connectivity in the case of functionalized and cross-linked porous structures.

**Phase II - 2018**

 In the second phase, new functionalization-crosslinking methods were developed and their efficiency was evaluated based on the thoroughly characterization of the thermal and mechanical properties as well as morphological features of the obtained 3D cellulose structures. A three-level organization in the 3D structures was detected by SEM investigation. A 5-fold increase in the specific compression and a minor decrease in thermal stability were observed depending on the cross-linking system. Acrylic copolymers were grafted on nanocellulose structures leading to a high improvement of properties: the increase of the contact angle from less than 30° to 84° indicating hydrophobic properties, reduced water absorption, increased onset degradation temperature with over 60 °C, a uniform polymer coating and a porous structure with high porosity. 3D composite structures based on nanocellulose and hydrophobic biopolymers, poly (3-hydroxybutyrate) (PHB), poly (3-hydroxybutyrate co-3-hydroxyvalerate) (PHBV) and poly (3-hydroxyoctanoate) were obtained by (i) impregnating functionalized nanocellulose structures with solutions of various biopolymers and (ii) mixing cellulosic nanofibres with these polymer solutions. Depending on the working conditions, the thermal stability of the composite structures increases by 100 °C compared to the untreated 3D cellulose structure. An extended porous network structure with pore sizes of less than 2 µm was observed on the surface of nanocellulose - PHBV nanocomposites modified by immiscible biopolymers. The report on the mechanical properties of the 3D crosslinked cellulose structures and the methodology for obtaining 3D structures from nanocellulose/hydrophilic polymers and from nanocellulose/hydrophobic biopolymers were released.

**Phase III - 2019**

 In the third phase, 3D porous structures were obtained from nanocellulose and biopolymers by (i) extrusion of nanocellulose mixtures with hydrophilic biopolymers and lyophilization, (ii) extrusion of nanocellulose mixtures with hydrophobic biopolymers and (iii) extrusion of mixtures of hydrophobic biopolymers, nanocellulose using blowing agents. The analysis of the correlations between the type of biopolymer, the process parameters and the characteristics of the nanocellulose and composite 3D structures allowed the selection of the representative porous structures for tissue engineering. The 3D nanocellulose structure model was developed. The potential cytotoxicity of surface-modified and cross-linked NC foams were evaluated using murine fibroblasts L929. *In vitro* analyzes showed that functionalized and cross-linked foams are not cytotoxic to L929 fibroblast cells, regardless of chemical treatment, which is very important for tissue engineering. The pro-inflammatory effect was also evaluated on the same 3D nanocellulose structures. NO and TNF-α were measured using the RAW 264.7 murine macrophage cell line to evaluate the influence of the chemical modifications on the activation of macrophage secretory function. TNF-α concentration was very low in the case of functionalized and cross-linked NC foams, similar to that of the negative control.

The results obtained in this project were disseminated by 8 ISI papers, 5 of which were published and three being in the evaluation and 2 non-ISI papers. The results were also disseminated in 21 international conferences, one being an invited lecture and two oral presentations.