SELECTED IN-VITRO STUDY OF BIONANOCELLULOSE TOWARD ITS UTILIZATION IN MEDICAL DEVICES CONTACTING WITH BLOOD

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Introduction

Prospectively, bionanocellulose (BNC) can be a widely applied biomaterial e.g. for manufacturing of dressings, contact lenses and implants like cartilages, valves or pericardial patches. BNC is a product of fermentation carried out by bacteria *Gluconacetobacter xylinus E25,* Cellulosic fibbers in diameter ap. 100um are cross-linked in the form of 3D nano-structure.

The goal of study was to assess the possibility of BNC application as a material to manufacturing medical devices permanently contacting with blood.

Materials and Methods

BNC was manufactured by Bowil Biotech company [1]. Raw material was delivered in the form of 10cm square patches of thickness about 1mm. Samples were lubricated by 0.9%NaCl and tightly wrapped.

Investigation of BNC focused on in-vitro biocompatibility study of raw material as well as assessment of functional properties of BNC in respect of its application in vascular grafts.

Concerning the biocompatibility study the following tests were carried out:

1) biodegradation test was carried out according to ISO10993-9 and -13 standard in formulation buffer pH=6.54 containing lysozyme with concentration of 2mg/ml (40000U/mg). Incubation was carried out by 30, 60 and 180 days in temperature of 37^{o}C. The degradation of material during incubation was assessed by means of TGA/DSC method and compared with reference material (no exposed to lysozyme). The morphology of surface was assessed by means of SEM (80Pa, 10kV).

2) thrombogenicity test was carried out according to own examination method based on Impact-R test and apparatus [2]. The idea of Impact-R is to assess the platelet (PLT) function under physiological-like shear stress generated by rotating cone. The 14.4mm of diameter BNC samples were contacted with 130ul of fresh human citrated blood for 300sec. at 720RPM of cone rotational speed. After circulation the evaluation of PLT activation and aggregation were assessed. Leucocytes and platelets were marked by utilizing CD45, CD62P and CD61 antibodies. Activated cells were measured by means of cytometry. Cells adhered to the surface of BNC samples were examined by means of fluorescent microscope. The reference was polystyrene. The positive control was polystyrene covered by Bionate 2 (manufactured by DSM) with manually rugged surface. Data were processed by means of ANOVA Kruskal-Wallis test and Mann-Whitney U-test.

Concerning the study of functional properties of BNC following tests were carried out:

3) permeability of BNC patches was assessed according to ISO 7198 standard on pure water and on porcine blood. Additionally, the permeability of commercial sealed graft (Gelveave/Vascutech and Intergard Knitted Graft sealed by means of gel and collagen, respectively) as well as unsealed graft (Bard type) was measured. Data were processed by means of ANOVA Kruskal-Wallis test and Mann-Whitney U-test.

4) athrombogenic features of grafts (3/8'' in diameter, 9cm of length,) made of BNC was examined based on acute thrombogenicity test [3] carried out on porcine blood anticoagulated by means of heparin. This qualitative test shows is device made of investigated biomaterial tend to form and adhere thrombus under continuous and pulsatile blood flow.

Results and Discussion

1) slightly decreasing of BNC thermal stability was observed which is related to formulation buffer migration insight the BNC fibre matrix. This thesis was confirmed in SEM examination with demonstrated buffer crystallites bounded to samples surface. In spite of minor decreasing of BNC thermal stability the material itself is resistant to biodegradation in lysozyme.

2) related the PLT activation there was no statistically signifficant differences between BNC, reference and positive control ($p>0.3299$). Related the PLT adhesion the comparison of pictures of samples revealed no differences between BNC and reference. The surface of positive controls was covered by numerous groups of aggregates. In respect of thrombogenicity BNC is equivalente to polystyrene.

3) mean permeability of BNC, gel-sealed, collagen-sealed and reference grafts in test carried out on blood were 0, 0, 2 and 26 ml/min/cm^2. In test carried out on water results were 0, 0, 5 and 196 ml/min/cm^2, respectively. Performed test showed, that BNC is impermeable for water as well as for blood.

4) in all cases spontaneous decreasing of ACT were occurred from ap. 260 s after heparin administration to ap. 140 s (end point of circulation). In two cases BNC surface contacted with flowing blood was free of visible clots formation. Immunohisochemical examination (eosin & haematoxylin dyeing) revealed insignificant fibrin layer with loosely bounded blood cells. In third case larger clots were formed as a result of ridged BNC surface. In all cases no hemolysis was found.

Conclusions

The BNC is resistant for enzymatic biodegradation. Its thrombogenicity is comparable to polystyrene. Material itself is impermeable for blood as well as for water with makes it suitable for manufacturing grafts and patches permanently contacted with blood. Additional examinations (e.g. animal trials) are necessary to confirm athrombogenic features of grafts made of BNC. It is essential to assess mechanical properties of BNC paying special attention to potential sphere of its application.

Acknowledgments

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