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The Biocompatibility of Membranes for Immunoisolation

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The assessment of biocompatibility of systems consisting of a host, permiselective membrane and a biological material, encapsulated within may be standardized for biological material immunoisolation purposes. The procedures for evaluation of the membrane non--toxicity against the biological material, the membrane physical and chemical stability post implantation and the biomaterial ability to perform with the host are presented.

K e y w o r d s: encapsulation, hollow fibers, membrane stability, membrane non-toxity, biomaterial performance with the host

1. Introduction

The classical definition of the biocompatibility is "the ability of a biomaterial to perform with an appropriate host response in a specific application" [1]. This definition offers no insights how to evaluate the biocompatibility or enhance it, however it is easy to interpret with the application of conventional artificial organs such as artificial knees or breasts, in the system consisting of the host and a biomaterial. The third element – a biological material encapsulated in a membrane makes the definition more complex. Generally, the biocompatible system of membranes is the system which neither evokes the host tissue overgrowth [2–5] nor exhibits the cytotoxic influence on the biological material.

The assessment of the biocompatibility of systems consisting of the host, a permiselective membrane, and a biological material encapsulated within may be standardized for biological material immunoisolation purposes. In our opinion to ensure the membrane biocompatibility analysis the following three steps of the membrane

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evaluation have to be included: (1) the membrane non-toxicity against the biological material; (2) the membrane physical and chemical stability post implantation; (3) the biomaterial ability to perform with the host, analyzed by microscopy of cells surrounding the membrane.

A review of the biocompatibility evaluation of the membranes performed in IBBE for implantable applications is presented.

2. Membrane Cytotoxity Evaluation

For a rational design of the biocompatibility testing, the procedure should relate to the future biomaterial application. The *in vitro* membrane non-cytoxicity for encapsulated biological material may be evaluated with different cell lines. Standards recommend, e.g. CCL cell line, but the biological material choice should be adequate for the further biomaterial application.

Different cell parameters may be evaluated, e.g. viability of cells, mitochondrial enzyme activity or DNA damage measurement [6]. In case of the biomaterials for direct contact with blood, the blood compatibility [7] is assessed by testing hemolytic action and coagulative and fibrinolytic parameters [8, 9] as well as the blood viscosity parameters [10] evaluation.

Cytocompatibility of the biomaterials for urinary catheters is evaluated using human urothelial cells [11]. For urinary reconstructions, evaluation using human bladder smooth muscle is recommended [12]. Cytotoxity of the biomaterials for oxygenators is evaluated on the human lung WI-38 cell line [13].

Several methods of the membrane cytotoxity evaluation with the membranes produced in our laboratories have been applied. These methods allow assessing cell life parameters of different cells. The Jurkat cell line may be applied as a model of human lymphocytes for immunoisolation device evaluation. As a model for differentiation of human hemopoietic cells the HL-60 and WEHI-3B mouse myelomonocyte cell line producing the IL-3 may be used. The cell life parameters like cell growth and viability were assessed.

In evaluation of the membranes cytotoxity, the polypropylene and polyethersulfone hollow fiber (HF) membranes with surface modified to improve their biocompatibility as potential prospects for implantable applications were used.

Table 1. The membranes applied for the cytotoxity evaluation

Hollow fiber	Parameters
PESf5 (produced in Dry Tests Lab. IBBE) Silanized	inner diameter 0.65 mm, wall thickness 0.04 mm
K600 (Membrana) silanized	inner diameter 0.60 mm, wall thickness 0.20 mm

The chosen cell lines were cultured in presence of the membrane material or encapsulated in the HF lumen and grown in culture media.

(1) The Jurkat cells were cultured in presence of the membrane material for 10 days. The results of evaluation of the Jurkat cells growth in ten days culture in the PESf5 silanized membrane presence were as follows [number of cells]: at day 1 $(1.0\pm0.1)\times10^6$, at day 5 $(2.0\pm0.06)\times10^6$, at day 10 $(3.0\pm0.15)\times10^6$. The number of cells increased during the cell culture 2-times after 5 days culture, 3-times after 10-days culture [14].

(2) The WEHI-3B cells encapsulated in the modified polypropylene membranes were cultured during 2 weeks. The WEHI-3B cells functioning was assessed non directly by evaluation of the BaF3 cells viability which depends on presence of the IL-3 (produced by the WEHI-3B cells) in the culture medium. The viability of the BaF3 cells cultured in presence of supernatant of the WEHI-3B culture was assessed in a flow cytometer (Fig. 1).

(3) The HL-60 cells encapsulated in the modified polypropylene membranes were cultured over 72 hours in the culture medium. The percentage of viable cells evaluated in flow cytometer was [%]: 32.0±1.41 for starting sample and 32.5±1.29 after 72-hour culture.

Considering the results obtained for the cytotoxity evaluation, it has been observed that neither the silanized poliethersulfone membranes nor the silanized polypropylene membranes influence the cells viability and the growth significantly.

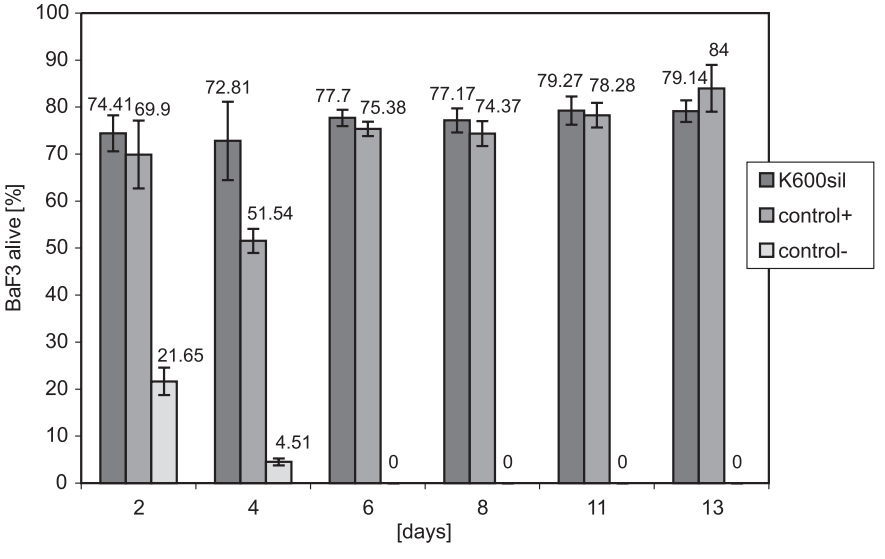

Fig. 1. Cytometric evaluation of the BaF3 cells viability, during the 24 h culture in presence of supernatant of the WEHI-3B cells encapsulated in HF for 2-, 4-, 6-, 8-, 11-, 13-day cultures. The viability of the BaF3 cells depends on presence of the IL-3 in the culture medium. As a positive control the supernatant of the nonencapsulated WEHI-3B cell culture was added to the BaF3 culture

Comparing the obtained results for the selected cell parameters: the ratio of the initial and final value for the viability evaluation of the HL-60 and WEHI-3B cells was respectively: 0.99 and 0.95.

Concluding, the membrane nontoxity to the biological material is one of the determinants of the biocompatibility. The obtained values depend on sort of the encapsulated biological material, the environment and the evaluation method, so the obtained values may be different, however the ratio of the measured parameters (except the cells growth) is close to 1 in the biocompatible system.

3. Evaluation of the Physical and Chemical Stability of the Membrane Material and its Ability to Perform with the Host

3.1. Membrane Physical and Chemical Stability Evaluation

The membrane physical and chemical stability should be included in the biocompatibility assessment. For this purpose the diffusive transport and FTIR were evaluated for the selected membranes. As the prospects for implantable applications the polypropylene K600 (Membrana, Germany) silanized OV1 (SERVA) or OV17 (SERVA) membranes were evaluated. The evaluated membranes were implanted subcutaneously into mice up to 4 months.

The diffusive transport evaluation seems to be an adequate method for physical stability evaluation of membranes for cell immunoisolation in the system HF-body fluids environment. The diffusive permeability was assessed using a thermodynamic description of diffusive mass transport across a homogenous membrane (Fick's law) and a two-compartment model.

The values of the diffusive membrane permeability [ml $min^{-1}m^{-2}$] obtained for large solute IgG, before and post implantation were respectively: 0.26±0.16 and 0.58±0.41 for OV1 membranes; 1.70±0.2 and 1.97±0.1 for OV17 membranes.

The FTIR evaluation may be representative for chemical stability assessment. Evaluation of the spectrum of absorption for infrared irradiation (Fourier Transformation Infrared, FTIR) is performed to evaluate the chemical stability of the membrane modification before and after implantation, using a FTS3000 MX (BioRad Excalibur, USA) device. The 16 scans were collected at resolution 4 cm^{-1} .

No changes during the FTIR evaluation of the membranes were observed up to 4 month implantation. The example picture of the FTIR spectrum for HF K600 OV1 post 4-months implantation is presented in Fig. 2. There is a characteristic peak for 1260 cm^{-1} wavenumber, indicating the presence of methyl group in bond with silicon.

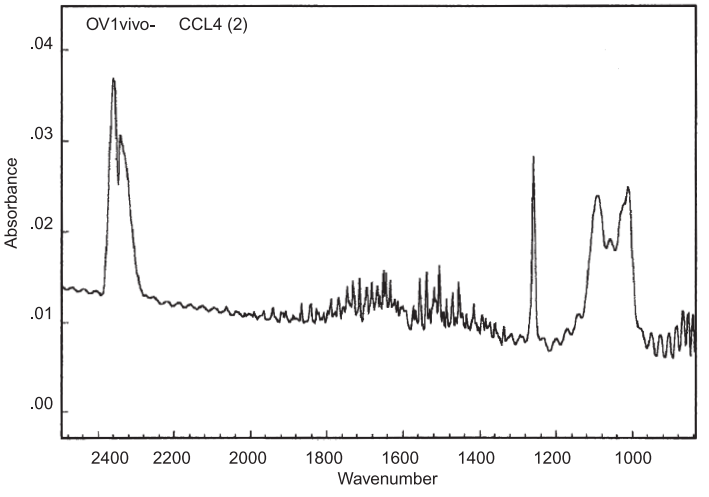

Fig. 2. The FTIR spectrum for OV1 silanized HF after 4 months implantation (the parallel – wave-number, the axial – absorbance)

The obtained diffusive permeability values were comparable before and post implantation, and there was no change found in the MWCO (molecular weight cut off) value. It may be concluded that the applied membranes ensure the physical stability.

Since no differences in the FTIR spectrum between K600 silanized OV1 or OV 17 before and post explantation up to 4 month of implantation are found, it is concluded, that the applied membranes provide a stable molecular structure [15].

3.2. Membrane Integrity Evaluation

The membrane material integrity may be evaluated during long – term implantation [16].

The polypropylene K600 HF silanized with different siloxanes were evaluated as the prospects for implantable applications (Table 2).

Table 2. The K600 different silanized membranes applied for the integrity evaluation

Hollow fiber	Polarity [%]
OV1 (SERVA)	25.8
OV7 (SERVA)	2.5
OV17 (SERVA)	1.0

The membranes of different polarity were implanted subcutaneously into mice for 6 month. The example effects of 6-months implantations are presented in the Fig. 3, 4.

No foreign body reaction was observed for all the evaluated membranes, however, there were differences in membrane integrity after implantation. The structures morphologically suggesting calcium salt were observed. For the OV1 membranes the calcium intrusions were observed on the external surface in maximal wall depth about 20 μm (Fig. 3). For the OV7 and OV17 membranes the calcium salt intrusions share was higher as compared to the OV1 membranes. The salt intrusions were found on the external membrane surface as well as in the membrane wall structure.

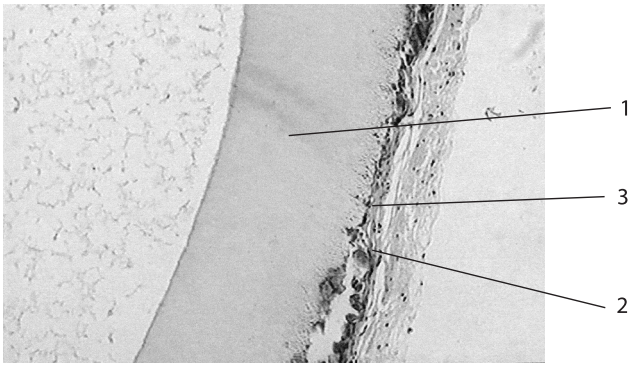

Fig. 3. The view of the K600 silanized OV1 after 6-months subcutaneous implantation into mouse. The external membrane wall is surrounded with a thin layer of fibroblasts. There are calcium salt intrusions in about 20 μm membrane wall thickness. No foreign body reaction was observed. 70×. 1 – membrane wall, 2 – fibroblasts layer, 3 – calcium salt intrusions. Magnification ×70

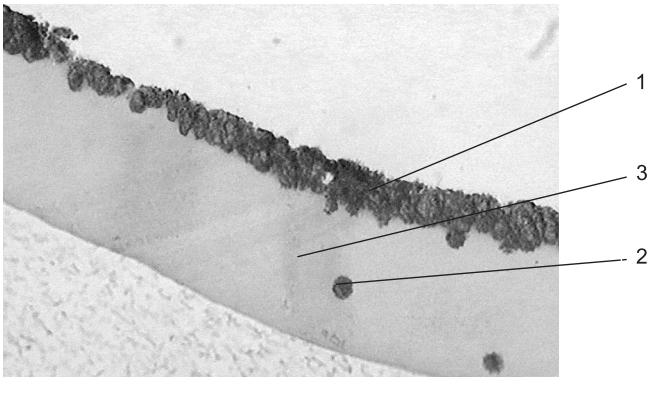

Fig. 4. The view of the K600 silanized OV7 after 6-months subcutaneous implantation into mouse. There are calcium salt intrusions in about 50 μm membrane wall thickness. No foreign body reaction was observed. 70×. 1, 2 – calcium salt intrusions, 3 – membrane wall. Magnification ×70

The different calcium salt intrusions presence in the different implanted membranes may be explained by different polarity of the evaluated membranes. Presuming the calcium salt intrusions share in the membrane equal or lower than 5% as

negligible, the trend line of power character ($y = 37.861x^{-0.6932}$) indicates that the membrane prospect for implantable applications should be the membrane of polarity not lower than 18.5% (Fig. 5).

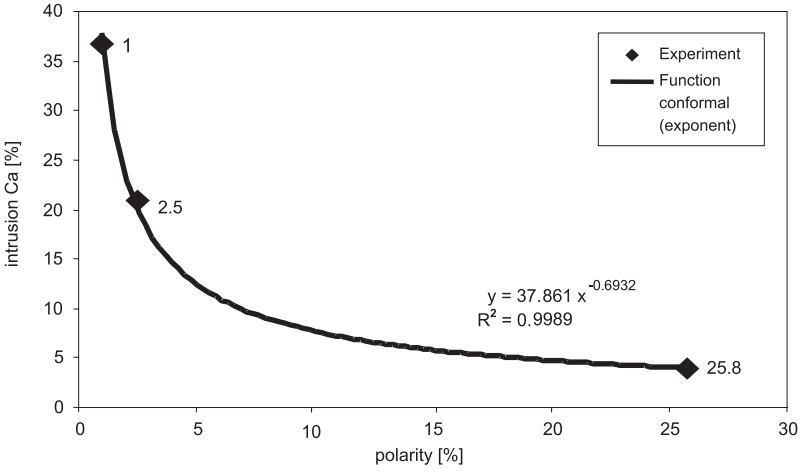

Fig. 5. Calcium salt dependence on the membrane polarity in subcutaneous implantation into the CDF1 mice

Evaluation of the membrane wall salt intrusions dependence on the membrane polarity is a quantitative criterion allowing for assessment of the membrane performance with the host and selection of the best biomaterial in terms of it's integrity.

3.3. Analysis of Biomaterial Ability to Perform with the Host Analyzed by Microscopy of Surrounding Cells

In this study, the membranes silanized with two selected siloxanes OV1 and OV17 were examined *in vivo* in subcutaneous implantations into mice up to 4 month, using light and electron microscopy evaluation.

The membrane silanized OV1 or OV17 may be observed by light microscopy after 2 or 4 months from implantation. The external surface of the evaluated HFs was surrounded by a thin layer of multinuclear cells and by the thin layer of fibroblasts. There was no inflammatory reaction with macrophages or lymphocyte infiltration behind the layer of fibroblasts which separate the membrane from the further host tissue.

The morphology of cells surrounding the silanized OV1 or OV17 membrane walls observed in electron microscopy was comparable for the selected days/months of observation. In the electron microscopy pictures, the silanized material can be seen to be covered with the host proteins some hours after implantation. Some inflammatory cells appear near the implanted membrane in a short time (4 days). However, no further presence of inflammatory cells is observed in 2 or 4 months beyond the

adherent collagen layer, near the external membrane wall. The membranes undergoing silanization by different applied siloxanes do not induce an in vivo massive tissue overgrowth and scar formation after implantation up to four months.

It may be concluded that the membranes tested in the experiment are biocompatible in the aspect of the membrane ability to perform with the host.

In other laboratories a range of commercially available hollow fiber membranes was evaluated for foreign body reaction and potential immunogenicity, in an effort to identify prospects for future implantable applications. Included among the hollow fiber membranes investigated is a wide variety of membranes representing several different materials and surface architecture. The samples were observed histologically by light microscopy to determine the presence and nature of the fibrotic capsule layer surrounding each type of membrane [17].

Table 3. Results of the membranes integrity and performance with the host evaluation in other laboratories [16]. For comparison the evaluation results of the polypropylene K600 (Membrana) modified in our laboratories for implantable purposes are included in the table

Membrane type	Composition	Pore size/ MWCO	Fibroblasts layer thickness after about 12-weeks implantation [μm]	Material integrity after implantation
Microfiltration	Mixed esters of cellulose (Spectrum Lab. Inc., USA)	0.2 μm	61	Preserved
	Polysulfone (A/G Technology, USA)	0.2 μm	51	Not preserved (membrane polymer particles presence in membrane surrounding tissue)
	Polysulfone (A/G Technology, USA)	0.65 μm	64	Not preserved (membrane polymer particles presence in membrane surrounding tissue)
	Polypropylene (Membrana, Germany) modified*	0.4 μm/ 200 kD	30	Preserved
Ultrafiltration	Polysulfone (Millipore Amicon)	10 kD	33	Preserved
	Regenerated cellulose (Spectrum Lab. Inc., USA)	13 kD	41	Preserved
Dialysis	Cellulose diacetate (Althin Medical USA)	68 kD	-	Degradation

* modification – IBIB Warsaw.

Owing to extensive degradation as early as 3 weeks, polysulfone microfiltration membranes and cellulose diacetate microdialysis membranes used in the other laboratories investigations were deemed unsuitable. Mixed esters of cellulose microfiltration membranes and microdialysis membrane in regenerated cellulose retained their integrity for duration of 12 week investigation. Polysulfone ultrafiltration membranes also remained intact for the duration of the implantation in the other laboratories evaluations as well as the polypropylene membranes modified and evaluated in our laboratories.

4. Conclusion

The presented evaluation of the membrane biocompatibility seems to be sufficient for identifying the potential prospect for implantable applications.

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