Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2021.26655

214 (2021) 135–145
February

Nanoencapsulation of neuronal cells for cryopreservation purposes

Anna Grzeczkowicz, Angelika Kwiatkowska, Monika Drabik, Ludomira Granicka*

Nalecz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, Trojdena 4 st., Warsaw, Poland, Tel. +48 22 592 59 00; emails: lgranicka@ibib.waw.pl (L. Granicka), agrzeczkowicz@ibib.waw.pl (A. Grzeczkowicz), ankwiatkowska@op.pl (A. Kwiatkowska), mdrabik@ibib.waw.pl (M. Drabik)

Received 26 March 2020; Accepted 25 May 2020

ABSTRACT

Cryopreservation is a commonly used method of long-term storage of biological material that guarantees the preservation of high genotypic stability of samples. It allows accumulating the required number of cells for successful transplant treatment, solving the problem of a limited number of cells isolated from the donor organs. Currently applied cryopreservation procedures have low efficiency and do not guarantee satisfactory cell viability after thawing. The study was aimed to construct and evaluate the polyelectrolyte multilayer's suitability in the cryopreservation of encapsulated neuronal cells. The multilayered membrane systems based on polyethyleneimine and polylysine or chitosan were constructed and analyzed. The designed systems were initially characterized using Fourier-transform infrared spectroscopy. The encapsulated cells underwent cryopreservation for up to 6 months. Then after thawing, were cultured for comparison in two different commercial culture media DMEM or Neurobasal® for 7 d. Cell function was examined using flow cytometry as well as mitochondrial activity assay. Besides, the morphology of the system membrane-cell after thawing was assessed using scanning electron microscopy. The proposed membrane systems were shown to be useful for cryopreservation of neuronal cells. However, the implementation of the modified procedure for routine preservation of clinical samples requires further studies.

Keywords: Nanoencapsulation; Polyelectrolyte multi-layered membranes; Cryopreservation; Neuronal cells

1. Introduction

The limitation of the number of cells isolated from the organs, necessary for effective transplantation therapy may be solved by the accumulation of it is an appropriate number. Thus, cell banking using cryopreservation is considered as a method allowing on-demand usage of long-term stored cells, for example, hepatocytes, pancreatic islets, erythrocytes or DNA need for transplantation therapies [1–5]. Cryopreservation enabling to store the cells for a proper time period gives the possibility to obtain the required amount of cells necessary for transplantation, however, the susceptibility of the cells isolated from organs to freezing damage might be a limitation. Encapsulation of cells within the protective membrane may be considered as a method allowing to protect cells during adverse freezing conditions, and sustain cell functions holding the promise for the continuous delivery of therapeutic agents [6–9]. Several encapsulation methods may be applied. However, the most popular is microencapsulation. This method proposed by Lim and Sun [10], has been the most studied and modified also for cryopreservation purposes [11], for example, the cryopreservation strategies for neural cells using neurospheres have been developed by many authors. Thus, microencapsulation in ultrahigh viscous

* Corresponding author.

alginate was proposed as the main strategy for avoiding the commonly observed neurosphere fragmentation and loss of cell–cell/matrix interactions post thawing. The method assures the availability of viable and functional stocks of neurospheres, thus reducing batch-to-batch variation [12]. Microencapsulation has a few limitations, one of them is the volume of encapsulated biological material necessary for effective transplantation.

The method of layer-by-layer deposition of polyelectrolytes (PE) for membrane forming can be chosen to ensure the optimal surface for exogenous primary neuronal cell growth using polymer self-assembly processes to form the PE multilayers. PE multilayer formation can be driven by different processes, including electrostatic interactions between oppositely charged constituents [13], hydrogen bonding [14–17], covalent bonding [18], and hydrophobic interactions [18,19]. The method allows for the multiple PE layers construction on biological material [20–22].

Dissociated cells from the central nervous system region contain neurons, astrocytes, oligodendrocytes, and other glial cells [23,24]. In contrast to glial cells, which easily adhere to many untreated surfaces, sensitive neurons require specific surfaces for the interface.

The polyelectrolyte membranes as substrates for neuronal cell growth have been under intense investigation [25–29]. The polyelectrolyte membranes used to form the layers may contain not only ionizable electrolyte groups but also additional functionality imparted by the polymer structure [30–35]. For instance, hydroxylated fullerene incorporated into PE multilayered membrane layers may increase hydrophilicity and improve layer stability [14].

Different materials of proven biocompatibility and/or biodegradability are typically used to form membrane layers. Among them, the following ones can be enumerated: polyethyleneimine, polylysine, and chitosan. These materials can be applied for cryopreservation.

The cryopreservation systems built of natural and synthetic polymer were applied in our investigations. Due to the unique multilayer membrane structure, these cryopreservation systems have the advantages of cell function sustaining.

In our study, the possibility of application of neural cells nano-coating and its protective role in cryopreservation of these cells was evaluated. Understanding the basis for the different approaches, it will be possible to select the best options of nano-coating for cell biopreservation, including neural cells, in different applications, and identify ways to improve preservation protocols for the future.

2. Experimental

2.1. Materials

2.1.1. Reagents

Chitosan from crab shells, polyethyleneimine (MW 60 kD), poly-L-lysine hydrobromide (MW15-30kD), hydrated polyhydroxy small gap fullerenes, bovine serum albumin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide mitochondrial activity assay (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Neurobasal medium, B27 supplement, glutamine, and glutamate were purchased from Life Technologies (Carlsbad, CA, USA), Dulbecco's Modified Eagle Medium (PAA, EU).

2.2. Methods

2.2.1. Primary neuronal cell culture

Cortical neuron cultures were prepared from the wastes of 19-day-old embryonic (E19) Wistar rat brains which isolation was as previously described [26]. Neurons were grown in Neurobasal® medium supplemented with 2% B27, 0.5 mM glutamine (Invitrogen, Carlsbad, CA, USA), 12.5 μM glutamate (Invitrogen), and a penicillin (100 U/mL)/streptomycin mixture (100 mg/mL) (Neurobasal) or in culture medium consisting of low glucose Dulbecco's Modified Eagle Medium containing 15% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin (Sigma) and HEPES buffer (1 mM) (PAA, EU) (DMEM).

The cells were cultured to over 90% confluence and then washed with Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+}, Mg^{2+}. After that, the cells were harvested with 0.25% trypsin EDTA (PAA). Cells were counted using a haemacytometer. After trypsinization, the cells were encapsulated within polyelectrolyte shells.

2.2.2. Membrane preparing

The following polyelectrolytes (PE) were used to build multilayer films: chitosan (CHIT), polyethyleneimine (PEI), poly-L-lysine (PLL). Solutions of PEI or PLL were prepared at a concentration 1 mg/mL in 0.1 M NaCl; alginate solution was prepared at a concentration 0.5 mg/mL in 0.1 M NaCl, and chitosan solution was prepared at a concentration 1 mg/mL in 2.5% (wt.) acetic acid. To obtain the polyethyleneimine (PEI) with fullerenol complex (PEI|FUOL), fullerenol solution in 0.1 M NaCl (pH 7.2) at a concentration of 0.5 mg/mL was added to PEI at a 1:10 ratio and subsequently stirred for 4 h at room temperature, as previously described [14]. The physicochemical properties of PEI|PLL, PEI|CHIT, PEI + FUOL|CHIT among other PE membranes using potential Zeta measurement, and AFM were previously reported [36].

2.2.3. Assessment of the transport properties of the membranes

The alginate cores prepared of 1.5% alginate solution in 0.1 M NaCl coated with the designed membranes were used to assess the membrane transport properties. The alginate cores served as a control. Diffusive permeability was evaluated using a thermodynamic description of diffusive mass transport across a homogenous membrane (Fick's law) and a two-compartment model [11]. Dextrans of molecular weight of 70 and 150 kDa were used as the model particle in these studies. Alginate cores coated with membranes were immersed in Dextran solution in physiological saline for 30 min to absorb the marker within the membrane. The cores were removed and Dextran concentration was measured. The difference between the initial concentration and the concentration after removing the coated cores was starting concentration in the process of

releasing a marker from the system. The coated alginate cores were immersed in physiological saline and then the concentration of Dextran was measured.

2.2.3.1. Fourier-transform infrared spectroscopy measurements

Evaluation of spectrum of absorption for red irradiation Fourier-transform infrared spectroscopy (FTIR) was performed using an FTS 3000MX (Bio Rad Excalibur, Hercules, CA, USA) device. FTIR curves were analyzed using Essential FTIR software. The presence of PE layers on the substrate was assessed by evaluating the spectrum of absorption for red irradiation (4,000–666 cm^{-1}) before culture. Liquid samples were contained in a KBr pellet, and 30 scans were typically performed at a resolution of 4 cm^{-1} and selectivity of 2 cm^{-1}.

2.2.4. Coating of cells with polyelectrolytes

5 × 10^6 of neural cells were incubated with PEI or PEI + FUOL solution at pH 7.2. After 6 min deposition time cells were washed 2-times with RPMI-1640 at 1,000 rpm for 3 min to remove unadsorbed polyelectrolyte. Encapsulated in one layer cells were incubated with the solution of PLL or CHIT at pH 7.2. After 4 min deposition time the coated cells were washed 2 times with RPMI-1640 at 1,000 rpm for 3 min to remove unadsorbed polyelectrolyte. Finally, the polyethyleneimine – polylysine (PEI|PLL), polyethyleneimine – chitosan (PEI|CHIT), polyethyleneimine with incorporated fullerenol-chitosan (PEI + FUOL|CHIT) bilayer was obtained.

2.2.5. Culture of encapsulated cells

The encapsulated neuronal cells were cultured (5% CO_2, 37°C) in Neurobasal or DMEM medium for 7 d, whereas half of the medium was changed every 3 d. As a negative control, neuronal cells were cultured (5% CO_2, 37°C) for 7 d. After that, the cells undergo cryopreservation. After cryopreservation, the cells were thawed. After thawing, the functioning of cells was examined using flow cytometry and MTT test after 24 h, 48 h and one week of culture in Neurobasal or DMEM medium. Scanning electron microscopy (SEM) using a Hitachi TM-1000 was used to evaluate the system morphology.

2.2.6. Cells banking in liquid nitrogen and thawing

The encapsulated or non-encapsulated (free) cells were subjected to cryopreservation according to standard, semi-controlled cooling, procedures. For the freezing procedure, the cells were detached from the plates and pelleted by centrifugation for 3 min at 1,300 rpm. Then, the pellet was suspended in the cryogenic medium supplemented with 20% DMSO (dimethyl sulfoxide; Sigma, EU). The culture was subjected to preliminary freezing at –80°C for 24 h and then placed into liquid nitrogen. The storage time in liquid nitrogen was 6 months.

For thawing, frozen cells were placed into the water bath at 37°C until ice dissolved. The thaw, free or encapsulated cells, were gently pipetted, washed twice with the culture medium and transferred to the culture plates. The non-encapsulated, non-cryopreserved and/or non-encapsulated thawed after cryopreservation cells served as a control.

2.2.7. Cell functioning analysis

2.2.7.1. Mitochondrial activity assay

The MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, UE) assay is based on the cleavage of yellow tetrazolium salt MTT to purple formazan crystals by metabolically active cells [17]. Non-encapsulated and encapsulated cells were seeded at a density of 1 × 10^4 cells per well in a 96-well plate. The solution of MTT at a concentration of 5 mg/mL was added to the culture at the ratio of 1:10 and incubated in a culture medium (5% CO_2, 37°C) for 2 h. Subsequently, the wells were centrifuged (1,600 rpm, 10 min) poured into the precipitate. Then 200 μl of DMSO was added for the formazan crystals dissolution. After 10 min of shaking the formazan absorbance was measured in a spectrophotometer at the wavelength 540 nm.

2.2.7.2. Flow cytometric analysis

The presence of cells was assessed using a Canto II flow cytometer (Becton Dickinson Immunocytochemistry Systems, USA). The results were processed by the FACS Diva software system (Becton Dickinson). Evaluated objects were separated from other events on light scatter characteristics.

2.2.8. SEM analysis

The cells, encapsulated within membranes, were visualized after thawing in the SEM; (TM 1000, Hitachi, Japan). The samples were dehydrated by the standard method, then a thin layer of gold was deposited on the cell surface by a sputtering system.

2.2.9. Statistical analysis

Mean values and standard deviations, as well as the significance of difference, were calculated in the Statistica 7.1 software. The values of $p < 0.05$ were assumed as significant.

3. Results and discussion

3.1. Assessment of the transport properties of the membranes

Analyzing the transport properties of evaluated membranes it was observed that the control group retained Dextran 70, allowing for about 32% of its release. The PEI|PLL, and PEI|CHIT allowed for 27% and 30% release respectively as related to the initial concentration value of 0.1 mg/mL.

On the other hand, PEI-F|CHIT allowed for less than 10% as related to the initial concentration value (Fig. 1) this is reflected in keeping permeability at a uniform level (Fig. 2).

The differences between the PEI-F|CHIT or other membranes may be caused due to the hydrogen interaction between NH groups of PEI and OH groups of fullerenol.

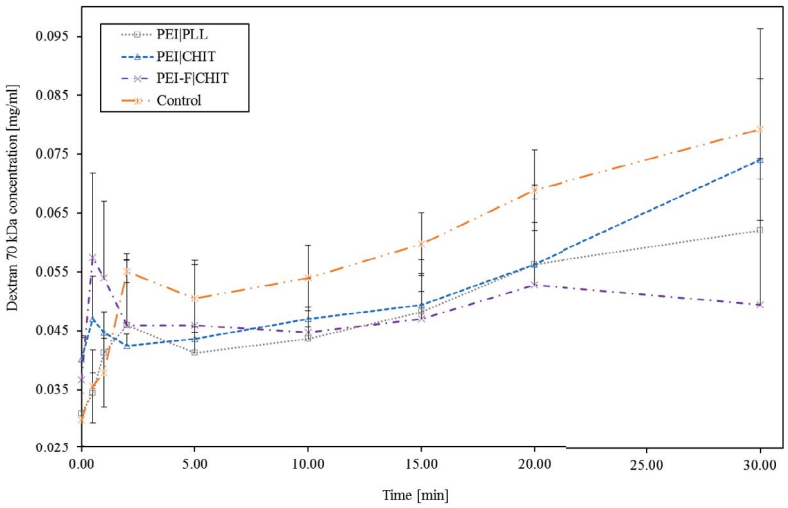

Fig. 1. Dextran 70 concentration as a function of time during permeation through the control or the membranes: PEI|PLL – polyethyleneimine|polylysine, PEI|CHIT – polyethyleneimine|chitosan, PEI-F|CHIT – polyethyleneimine with incorporated fullerenol|chitosan. The values are presented as mean ±SD.

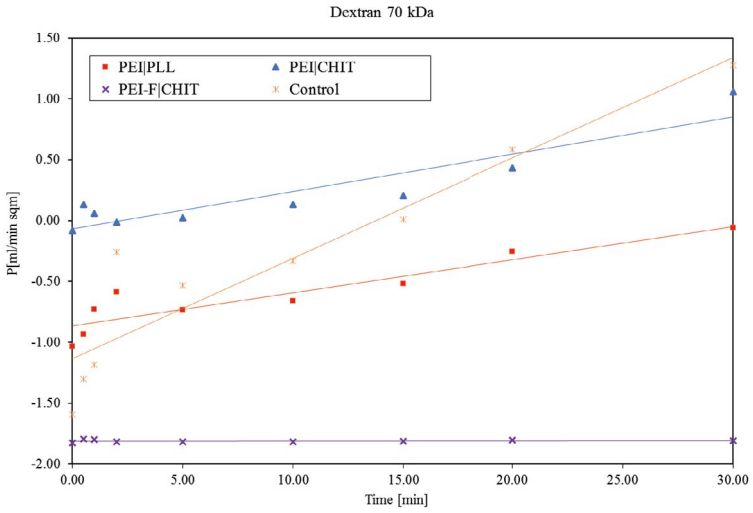

Fig. 2. Permeability for Dextran 70 as a function of time for the control or the membranes: PEI|PLL – polyethyleneimine|polylysine, PEI|CHIT – polyethyleneimine|chitosan, PEI-F|CHIT – polyethyleneimine with incorporated fullerenol|chitosan. The values are presented as mean ±SD.

Some of the OH groups of chitosan remained uninvolved in the interaction with PEI, which might allow for increased attractive interaction with Dextran OH groups.

The control and all evaluated membranes retained Dextran 150 allowing for lower than 10% release after 30 min as related to the starting concentration value 0.1 mg/mL (Fig. 3).

The obtained values indicated that the membrane cut-off was on the 150 kDa level for PEI|PLL, PEI|CHIT. On the other hand, the membrane PEI-F|CHIT cut-off was at albumin level value (Fig. 4).

3.2. Fourier-transform infrared spectroscopy

Fourier transform infrared spectroscopy analysis allowed to assess the presence of substrates forming the membrane layers (Fig. 5). For PEI|PLL membrane the layer presence was confirmed by characteristic peaks at the following frequencies (cm^{-1}): 3,375 and 1,631 representing N–H stretching vibrations in PEI and PLL; Also, for PEI|CHIT membrane the presence of the layer was detected by characteristic peaks at the following frequencies (cm^{-1}): 3,375, representing O–H stretching vibrations in CHIT, N–H stretching vibrations in CHIT, PEI and PLL; 1,631, representing O–H stretching vibrations in CHIT, N–H, stretching vibrations in CHIT, PEI and PLL;

Finally, for PEI-F|CHIT membrane the layer presence was confirmed by characteristic peaks at the following frequencies (cm^{-1}): 3,375, representing O–H stretching vibrations in CHIT, N–H stretching vibrations in CHIT, PEI and PLL; 1,631, representing O–H stretching vibrations in CHIT, N–H, stretching vibrations in CHIT, PEI and PLL.

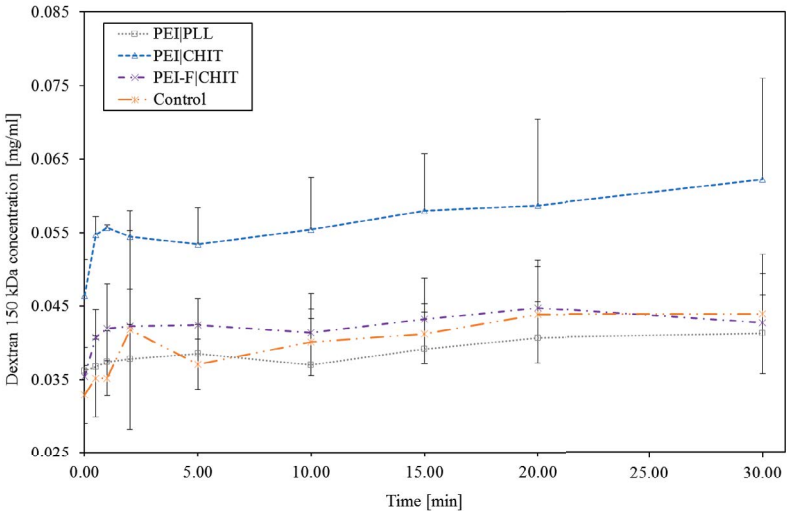

Fig. 3. Dextran 150 concentration as a function of time during permeation through the control or the membranes: PEI|PLL – polyethyleneimine|polylysine, PEI|CHIT – polyethyleneimine|chitosan, PEI-F|CHIT – polyethyleneimine with incorporated fullerenol|chitosan. The values are presented as mean ±SD.

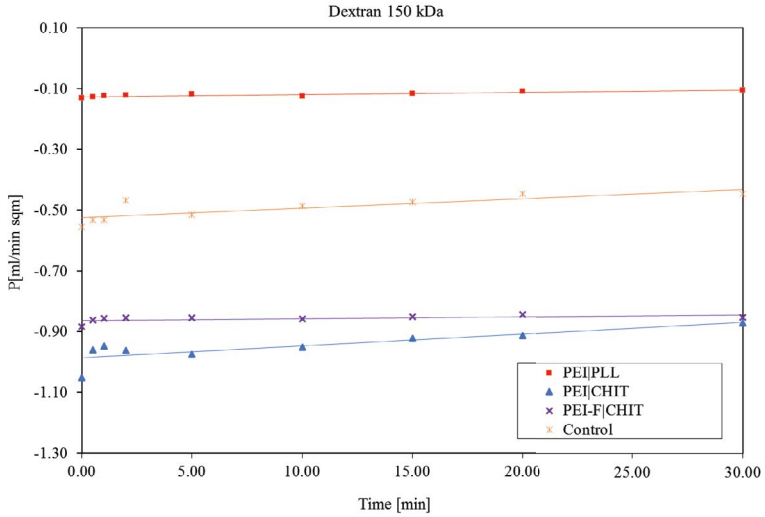

Fig. 4. Permeability for Dextran 150 as a function of time for the control or the membranes: PEI|PLL – polyethyleneimine|polylysine, PEI|CHIT – polyethyleneimine|chitosan, PEI-F|CHIT – polyethyleneimine with incorporated fullerenol|chitosan. The values are presented as mean ±SD.

It can be noted that the C–C bands of fullerenol at about 1,600 and 1,147 are not developed. It can be caused by a strong PEI|CHIT interaction presence.

It can be mentioned that the picks at 3,375 and 1,631 overlapped for NH groups as well as OH groups in different applied membranes.

3.3. Evaluation of the functioning of cells within different membranes

3.3.1. Mitochondrial activity of neural cells

The impact of membranes constructed on the cells surface and the influence of culture medium applied in the culture on the functioning of the thawed neural cells was assessed. It should be noted that the cells were cultured before freezing and after thawing in two different media: Neurobasal or DMEM.

The figures below present the results obtained for the comparison between the membrane coatings.

The analysis of mitochondrial activity showed that there was a statistical difference in the values obtained for the cells encapsulated in PEI-F|CHIT comparing with the cells uncoated (control) or coated with other membranes after 7 d of the culture in DMEM (Fig. 6).

There was a statistical difference in the values obtained for the cells encapsulated in PEI-F|CHIT comparing with the cells uncoated (control) or coated with membranes PEI|PLL, PEI|CHIT during 7 d culture in Neurobasal medium. The cells encapsulated within the PEI-F|CHIT membrane exhibited the highest mitochondrial activity during the whole period of culture (Fig. 7).

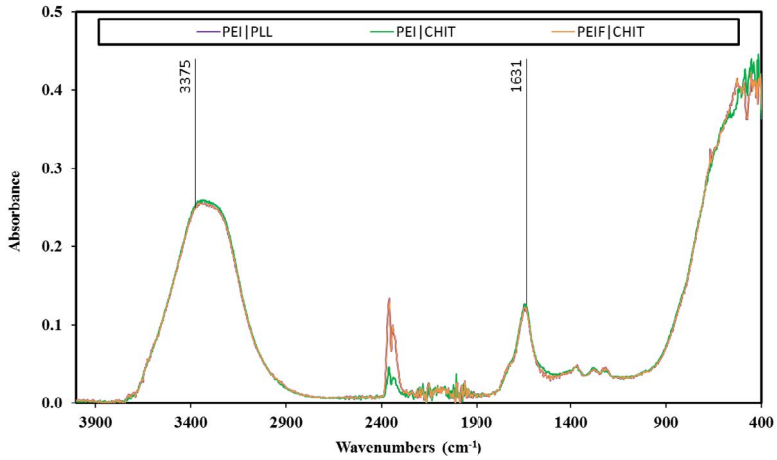

Fig. 5. FTIR spectrum of PEI|PLL, PEI|CHIT, and PEIF|CHIT membranes.

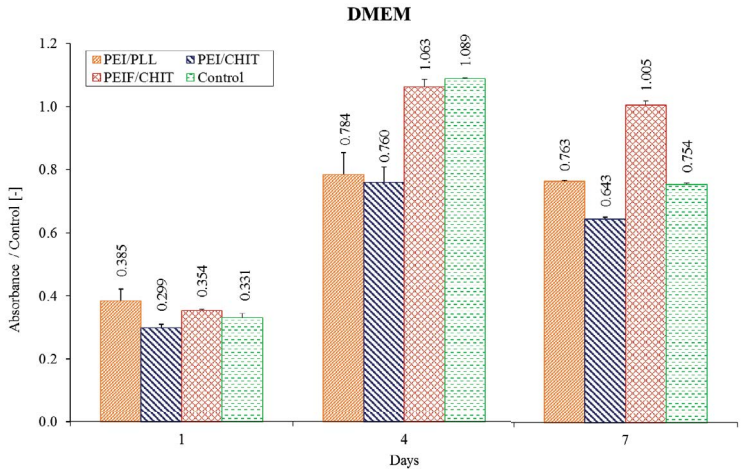

Fig. 6. The ratio of the mitochondrial activity of neural cells after thawing non encapsulated or encapsulated to the non-encapsulated not undergone cryopreservation cells, expressed by formazan production during 7 d culture in DMEM. Membranes polyethyleneimine|polylysine (PEI|PLL), polyethyleneimine|chitosan (PEI/CHIT), polyethyleneimine with incorporated fullerenol|chitosan (PEI-F|CHIT). The values are presented as the mean ±SD (n = 6).

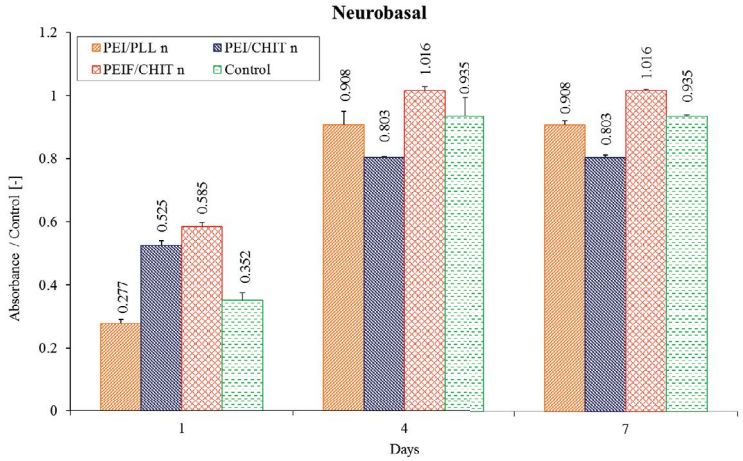

Fig. 7. The ratio of the mitochondrial activity of neural cells after thawing non encapsulated or encapsulated to the non-encapsulated not undergone cryopreservation cells expressed by formazan production, during 7 d culture in Neurobasal. Membranes polyethyleneimine|polylysine (PEI|PLL), polyethyleneimine|chitosan (PEI|CHIT), polyethyleneimine with incorporated fullerenol|chitosan (PEI-F|CHIT). The values are presented as the mean ±SD (n = 6).

Analyzing the influence of the time of culture maintained in DMEM after cells thawing, a general upward trend in mitochondrial activity on 4-, 7 d of culture was noticed, comparing with 1st day of culture (Fig. 8). There was no statistical difference between the cells coated with PEI|PLL after 4- and 7 d culture. There was a statistical difference in mitochondrial activity of cells coated with PEI|CHIT or PEI-F|CHIT on the 4th day of culture compared with the 7th day of culture. However, a decline on the 7th day was maximally up to 15%. Whereas the control value diminished by about 30%.

Also, the analysis of the influence of the time of culture in Neurobasal medium after cell thawing exhibited a general upward trend in mitochondrial activity on 4-, 7 d of culture compared with the 1st day of culture (Fig. 9).

There was no statistical difference on the 4th and 7th day of culture in Neurobasal between the cells uncoated or coated with PEI|PLL or PEI|CHIT or PEI-F|CHIT (Fig. 4). It can be noted that the Neurobasal ensures more stable values of mitochondrial activity during the 4- or 7 d culture comparing with the DMEM medium.

3.3.2. *Viability of neural cells*

Analyzing the viability of cells coated within individual membranes cultured in DMEM, the significant decline on the 4th and 7th day of culture comparing with 1st day was observed in the case of cells coated with PEI|PLL, and PEI|CHIT, whereas the significant increase was noticed for PEI-F|CHIT on the 7th day (Figs. 10 and 11). The viability

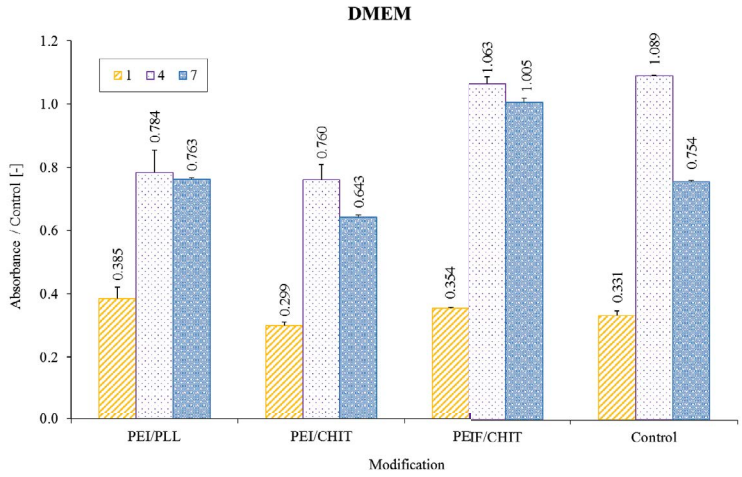

Fig. 8. The ratio of the mitochondrial activity of neural cells after thawing non encapsulated or encapsulated to the non-encapsulated not undergone cryopreservation cells, expressed by formazan production during 7 d culture in DMEM. Membranes polyethyleneimine|polylysine (PEI|PLL), polyethyleneimine|chitosan (PEI/CHIT), polyethyleneimine with incorporated fullerenol|chitosan (PEI-F|CHIT). The values are presented as the mean ±SD (n = 6).

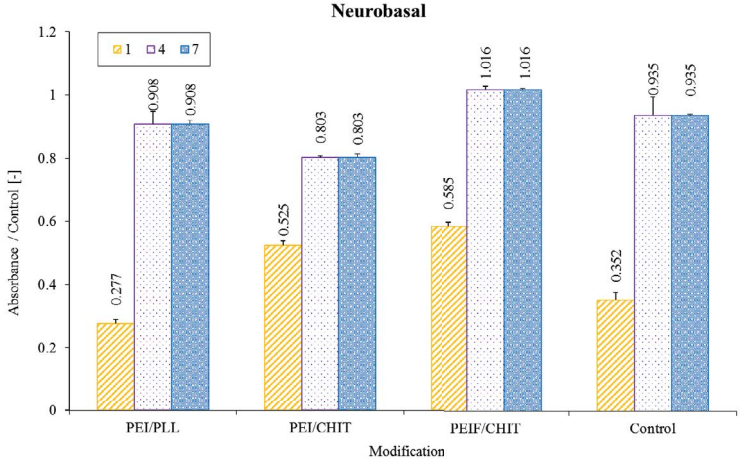

Fig. 9. The ratio of the mitochondrial activity of neural cells after thawing non encapsulated or encapsulated to the non-encapsulated not undergone cryopreservation cells expressed by formazan production, during 7 d culture in Neurobasal. Membranes polyethyleneimine|polylysine (PEI|PLL), polyethyleneimine|chitosan (PEI|CHIT), polyethyleneimine with incorporated fullerenol|chitosan (PEI-F|CHIT). The values are presented as the mean ±SD (n = 6).

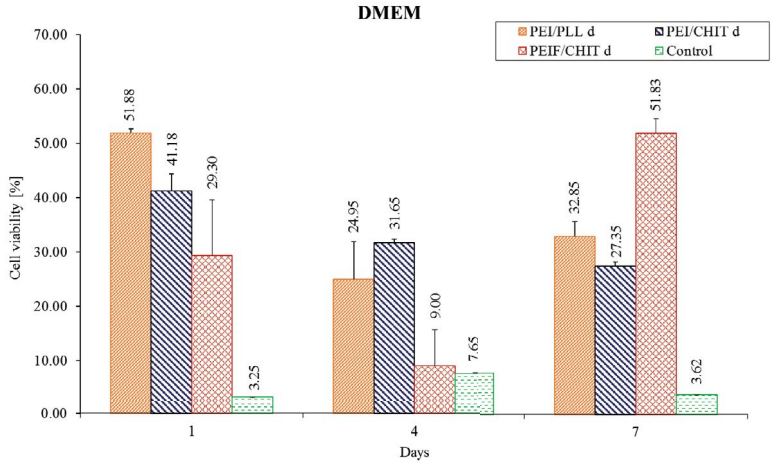

Fig. 10. The viability of neural cells after thawing, nonencapsulated (control) or encapsulated within the membranes: polyethyleneimine|polylysine (PEI|PLL), polyethyleneimine|chitosan (PEI/CHIT), polyethyleneimine with incorporated fullerenol|chitosan (PEI-F|CHIT) during 7 d culture in DMEM. The values are presented as the mean ±SD (n = 6).

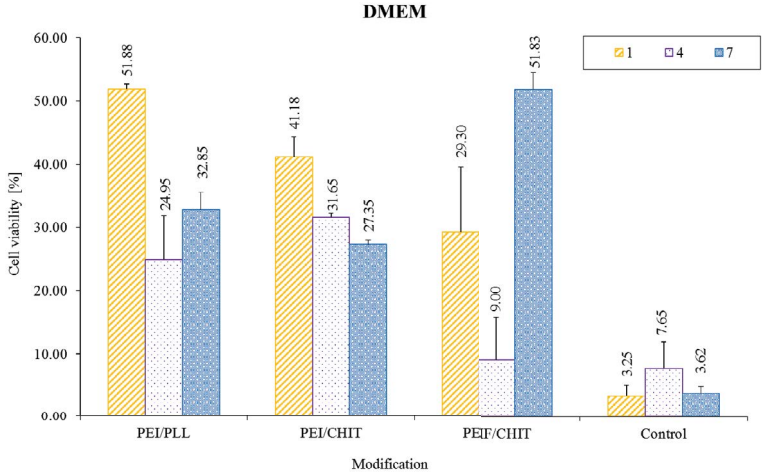

Fig. 11. The viability of neural cells after thawing, nonencapsulated (control) or encapsulated within the membranes: polyethyleneimine|polylysine (PEI|PLL), polyethyleneimine|chitosan (PEI/CHIT), polyethyleneimine with incorporated fullerenol|chitosan (PEI-F|CHIT) during 7 d culture in DMEM. The values are presented as the mean ±SD (n = 6).

of control cells was meanly 4.8 ± 2.4 during the 7 d culture (Fig. 10).

The analysis of the percentage of the viable cells in the population of non-encapsulated or encapsulated cells during the culture in Neurobasal medium after thawing showed no statistical differences between the values obtained for the cells encapsulated in PEI|PLL, PEI|CHIT and PEI-F|CHIT after 7 d (Fig. 12). Wherein, the noncoated viable cells share during the 7 d culture was meanly 7.4 ± 1.5 and was significantly lower compared with the values obtained for cells coated with different membranes. There was no statistical difference between the viable cells share after the 4th day and 7th day of culture encapsulated with PEI|PLL or PEI-F|CHIT or PEI|CHIT.

Analyzing the viability of cells coated within individual membranes cultured in Neurobasal, no significant changes were observed after 7 d of culture compared with the 1st day in the case of membrane coating built with PEI|CHIT, and PEI-F|CHIT (Fig. 13). It can be noted that the steadiest values during 7 d culture were observed for the cells encapsulated within PEI|PLL (Fig. 13). The mitochondrial activity of encapsulated cells was the highest in the case of PEI-F|CHIT membrane for the 7th-day culture in both types of culture media. Moreover, the viable cells share was the highest in the case of PEI-F|CHIT membrane after 7 d culture in DMEM. However, the viability values were differential during the time of culture. Nevertheless, the viability of encapsulated in different membranes cells was comparable after 7 d culture in Neurobasal. The values obtained for different culture media indicated the possibility of using both media Neurobasal and DMEM before cryopreservation and after thawing.

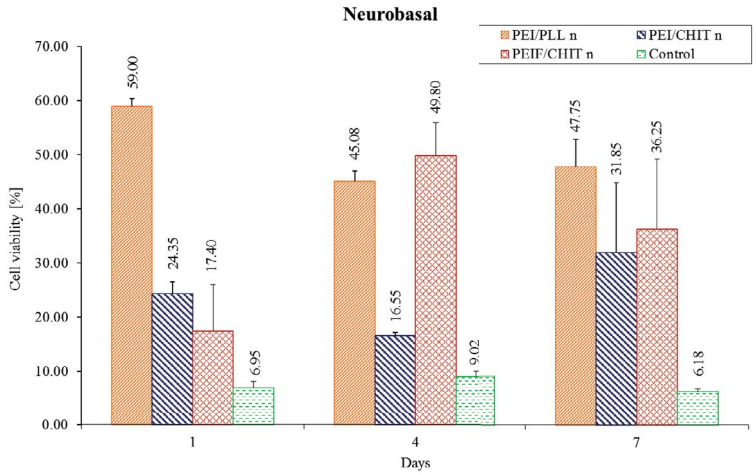

Fig. 12. The viability of neural cells after thawing, nonencapsulated (control) or encapsulated within the membranes: polyethyleneimine|polylysine (PEI|PLL), polyethyleneimine|chitosan (PEI|CHIT), polyethyleneimine with incorporated fullerenol|chitosan (PEI-F|CHIT) during 7 d culture in Neurobasal. The values are presented as the mean ±SD (n = 6).

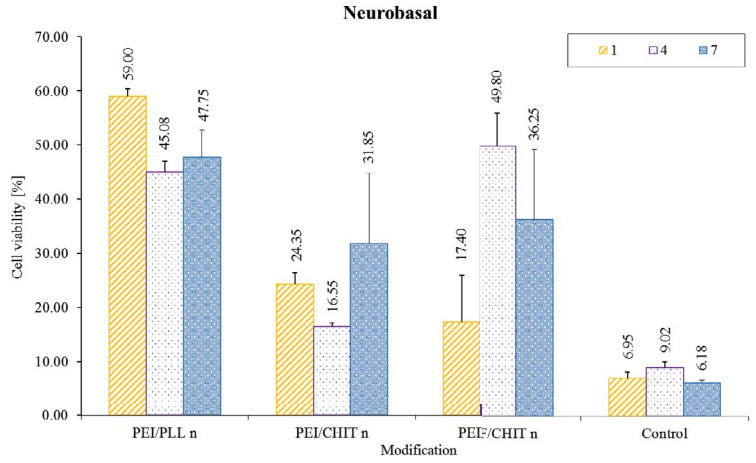

Fig. 13. The viability of neural cells after thawing, nonencapsulated (control) or encapsulated within the membranes: polyethyleneimine|polylysine (PEI|PLL), polyethyleneimine|chitosan (PEI/CHIT), polyethyleneimine with incorporated fullerenol|chitosan (PEI-F|CHIT) during 7 d culture in Neurobasal. The values are presented as the mean ±SD (n = 6).

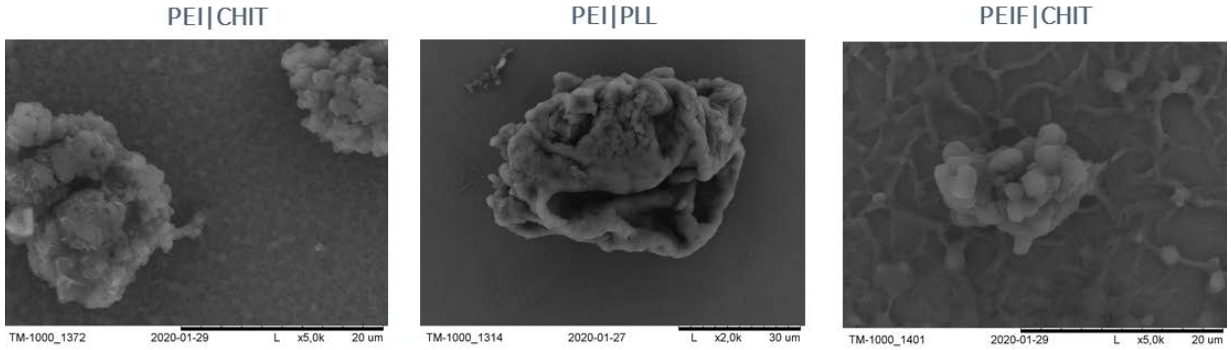

Fig. 14. SEM visualization of neural cells encapsulated in designed membranes after thawing and 24 h of the culture. Polyethyleneimine|polylysine (PEI|PLL), polyethyleneimine|chitosan (PEI|CHIT), polyethyleneimine with incorporated fullerenol|chitosan (PEI-F|CHIT).

3.4. SEM analysis

To assess the morphology of the systems consisted of the cells coated with the membrane shells, SEM analysis was applied. The exemplary pictures of the thawed cells coated with chosen membranes, after the 24 h of culture, are presented in Fig. 14.

All the systems of encapsulated cells showed spherical shape however differed in morphology. The systems using PEI|CHIT and PEI-F|CHIT presented a spongy structure, whereas the PEI|PLL share revealed a more compact structure. Moreover, the system based on PEI-F|CHIT tends to open the structure allowing the encapsulated cells to expand beyond the membrane.

4. Conclusion

We applied the polyelectrolyte membrane material for the coating of neural cells for cryopreservation purposes. Although the control activity is at a level comparable to coated cells, the percentage of live cells is lower as compared to the coated cells. Thus, the coating application is justified. All evaluated membranes can be applied for cryopreservation purposes. They can be recommended for biomedical applications as resistant material ensuring the maintenance of the cell function after 6 months of cryopreservation.

References

[1] S. Mansouri, Y. Merhi, F.M. Winnik, M. Tabrizian, Investigation of layer-by-layer assembly of polyelectrolytes on fully functional human red blood cells in suspension for attenuated immune response, Biomacromolecules, 12 (2011) 585–592.

[2] P.B. Stiegler, V. Stadlbauer, S. Schaffellner, G. Halwachs, C. Lackner, O. Hauser, F. Iberer, K. Tscheliessnigg, Cryopreservation of insulin-producing cells microencapsulated in sodium cellulose sulfate, Transplant. Proc., 36 (2006) 3026–3030.

[3] Y. Teramura, H. Iwata, Bioartificial pancreas microencapsulation and conformal coating of islet of Langerhans, Adv. Drug Delivery Rev., 62 (2010) 827–840.

[4] K. Tatsumi, K. Ohashi, Y. Teramura, R. Utoh, K. Kanegae, N. Watanabe, S. Mukobata, M. Nakayama, H. Iwata, T. Okano, The non-invasive cell surface modification of hepatocytes with PEG-lipid derivatives, Biomaterials, 33 (2012) 821–828.

[5] A.C. Dorris, K.L. Douglas, M. Tabrizian, C.J. Barrett, Control of DNA incorporation into nanoparticles with poly(L-lysine) multilayers, Can. J. Chem., 86 (2008) 1085–1094.

[6] K.-C. Yang, C.-C. Wu, Z.-F. Kuo, C.-Y. Yang, F.-H. Lin, Intramedullary cavity as implantation site for bioartifical pancreas: preliminary in vivo study, Transplant. Proc., 42 (2010) 2666–2668.

[7] Y. Yang, J.Z. Li, X.P. Pan, P.C. Zhou, X.P. Yu, H.C. Cao, Y.J. Wang, L.J. Li, Co-culture with mesenchymal stem cells enhances metabolic functions of liver cells in bioartificial liver system, Biotechnol. Bioeng., 110 (2013) 958–968.

[8] A. Murua, A. Portero, G. Orive, R.M. Hernández, M. de Castro, J.L. Pedraz, Cell microencapsulation technology: towards clinical application, J. Controlled Release, 132 (2008) 76–83.

[9] J.A. Baddour, K. Sousounis, P.A. Tsonis, Organ repair and regeneration: an overview, Birth Defects Res. C Embryo Today, 96 (2012) 1–29.

[10] F. Lim, A. Sun, Microencapsulated islets as bioartificial endocrine pancreas, Science, 210 (1980) 908–910.

[11] C.C. Zhang, Y.L. Zhou, L. Zhang, L. Wu, Y.J. Chen, D. Xie, W.Y. Chen, Hydrogel cryopreservation system: an effective method for cell storage, Int. J. Mol. Sci., 19 (2018) 3330.

[12] R. Malpique, L.M. Osório, D.S. Ferreira, F. Ehrhart, C. Brito, H. Zimmermann, P.M. Alves, Alginate encapsulation as a novel strategy for the cryopreservation of neurospheres, Tissue Eng. Part C, 16 (2010) 965–977.

[13] R.K. Iler, Multilayers of colloidal particles, J. Colloid Interface Sci., 21 (1966) 569–594.

[14] M. Borkowska, E. Godlewska, M. Antosiak-Iwańska, J. Kinasiewicz, M. Strawski, M. Szklarczyk, L.H. Granicka, Suitability of polyelectrolyte shells modified with fullerene derivate for immunoisolation of cells. Experimental study, J. Biomed. Nanotechnol., 8 (2012) 1–6.

[15] S.L. Clark, P.T. Hammond, The role of secondary interactions in selective electrostatic multilayer deposition, Langmuir, 16 (2000) 10206–10214.

[16] Y. Fu, S.L. Bai, S.X. Cui, D.L. Qiu, Z.Q. Wang, X. Zhang, Hydrogen-bonding-directed layer-by-layer multilayer assembly: reconformation yielding microporous films, Macromolecules, 35 (2002) 9451–9458.

[17] E. Kharlampieva, V. Kozlovskaya, J. Tyutina, S.A. Sukhishvili, Hydrogen-bonded multilayers of thermoresponsive polymers, Macromolecules, 38 (2005) 10523–10531.

[18] Y. Teramura, H. Iwata, Cell surface modification with polymers for biomedical studies, Soft Matter, 6 (2010) 1081–1091.

[19] N.A. Kotov, Layer-by-layer self-assembly: the contribution of hydrophobic interactions, Nanostruct. Mater., 12 (1999) 789–796.

[20] M. Bulwan, M. Antosiak-Iwańska, E. Godlewska, L. Granicka, S. Zapotoczny, M. Nowakowska, Chitosan-based nanocoatings for hypothermic storage of living cells, Macromol. Biosci., 13 (2013) 1610–1620.

[21] T. Bhaiji, Z.-L. Zhi, J.C. Pickup, Improving cellular function and immune protection via layer-by-layer nanocoating of pancreatic islet β-cell spheroids cocultured with mesenchymal stem cells, J. Biomed. Mater. Res. Part A, 100 (2012) 1628–1636.

[22] S. Kizilel, A. Scavone, X. Liu, J.-M. Nothias, D. Ostrega, P. Witkowski, M. Millis, Encapsulation of pancreatic islets within nano-thin functional polyethylene glycol coatings for enhanced insulin secretion, Tissue Eng. Part A, 16 (2010) 2217–2228.

[23] B.E. Reubinoff, P. Itsykson, T. Turetsky, M.F. Pera, E. Reinhartz, A. Itzik, T. Ben-Hur, Neural progenitors from human embryonic stem cells, Nat. Biotechnol., 19 (2001) 1134–1140.

[24] Q.-L. Cao, Y.P. Zhang, R.M. Howard, W.M. Walters, P. Tsoulfas, S. Whittemore, Pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord are restricted to a glial lineage, Exp. Neurol., 167 (2001) 48–58.

[25] I.-C. Lee, Y.-C. Wu, Facilitating neural stem/progenitor cell niche calibration for neural lineage differentiation by polyelectrolyte multilayer films, Colloids Surf., B, 121 (2014) 54–65.

[26] K. Zhou, G.Z. Sun, C.C. Bernard, G.A. Thouas, D.R. Nisbet, J.S. Forsythe, Optimizing interfacial features to regulate neural progenitor cells using polyelectrolyte multilayers and brain derived neurotrophic factor, Biointerphases, 6 (2011) 189.

[27] C. Vodouhê, M. Schmittbuhl, F. Boulmedais, D. Bagnard, D. Vautier, P. Schaaf, C. Egles, J.-C. Voegel, J. Ogier, Effect of functionalization of multilayered polyelectrolyte films on motoneuron growth, Biomaterials, 26 (2005) 545–554.

[28] S.M. Ryu, B.-S. Kim, Culture of neural cells and stem cells on graphene, J. Tissue Eng. Regener. Med., 10 (2013) 39–46.

[29] H.-W. Liu, W.-C. Huang, C.-S. Chiang, S.-H. Hu, C.-H. Liao, Y.-Y. Chen, S.-Y. Chen, Arrayed rGO_{SH}/PMA_{SH} microcapsule platform integrating surface topography, chemical cues, and electrical stimulation for three-dimensional neuron-like cell growth and neurite sprouting, Adv. Funct. Mater., 24 (2014) 3715–3724.

[30] S. Pavlukhina, S. Sukhishvili, Polymer assemblies for controlled delivery of bioactive molecules from surfaces, Adv. Drug Delivery Rev., 63 (2011) 822–836.

[31] Y. Teramura, Y. Kaneda, H. Iwata, Islet-encapsulation in ultra-thin layer-by-layer membranes of poly(vinyl alcohol) anchored to poly(ethylene glycol)–lipids in the cell membrane, Biomaterials, 28 (2007) 4818–4825.

[32] J. Sun, Y.B. Huang, Q. Shi, X. Chen, X.B. Jing, Oxygen carrier based on hemoglobin poly($_L$-lysine)-*block*-poly($_L$-phenylalanine) vesicles, Langmuir, 25 (2009) 13726–13729.

[33] J.E. Wong, H. Zastrow, W. Jaeger, R. Von Klitzing, Specific ion versus electrostatic effects on the construction of polyelectrolyte multilayers, Langmuir, 25 (2009) 14061–14070.
[34] H.T. Cui, J. Shao, Y. Wang, P.B. Zhang, X.S. Chen, Y. Wei, PLA-PEG-PLA and its electroactive tetraaniline copolymer as multi-interactive injectable hydrogels for tissue engineering, Biomacromolecules, 14 (2013) 1904–1912.
[35] K.S. Schanze, A.H. Shelton, Functional polyelectrolytes, Langmuir, 25 (2009) 13698–13702.
[36] A. Grzeczkowicz, J. Gruszczyńska-Biegala, M. Czeredys, A. Kwiatkowska, M. Strawski, M. Szklarczyk, M. Koźbiał, J. Kuźnicki, L.H. Granicka, Polyelectrolyte membrane scaffold sustains growth of neuronal cells, J. Biomed. Mater. Res. Part A, 107 (2019) 839–850.