PREPARATION OFAMPHIPHILIC PDMS BLOCK COPOLYMER NANOMATERIALSCONTAINING PVPFOR BIOMEDICAL APPLICATION

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ABSTRACT

In this study, we present PDMS-based amphiphilic block copolymers containing PVP (Poly N-vinyl pyrrolidone) hydrophilic polymer block with different chain length. The amphiphilic copolymers are synthetized by conventional radical polymerization initiated with rarely used PDMS-macroazoinitiator. From these block copolymers we prepared nano-containers (polymersomes) for drug delivery application, accomplished by film rehydration, solvent evaporation and extrusion methods and nano ranged 3D amphiphilic polymer scaffolds, using versatile technique such as electrospinning. PDMS-based amphiphilic block copolymers containing PVP (Poly N-vinyl pyrrolidone)were studied for biological response: cell proliferation, cell adhesion and morphology after contact with polymer surfaces. The obtained materials are biocompatible, due to the optimal chain length of hydrophilic blocks, pore size and roughness of polymer materials based of polymerosones. All materials are characterized by different spectroscopic and microscopic techniques.

***Key words**: PDMS, PVP,macroazoinitiator (MAI), diblock copolymer, SEM, XPS, DLS, polymeric vesicles,electrospinning, biocompatibility, drug delivery*

Introduction

Polydimethylsiloxane (PDMS) is one of the most interesting inorganic polymer material, which is used for different application, for example, medical devices, surgery, drug delivery systems and pharmaceutical ingredients (1-6).PDMS is inorganic biocompatible polymer mostly due to the chemical and physiological inertness (7, 8). Its physic-chemical characteristics as flexibility, very low glass transition temperature, low surface energy, tension and solubility (8), excellent film forming properties attract the scientific attention and triggering the investigation and the preparation of amphiphilic block copolymers.The silicone-specific features of this polymer in effective combination with other polymer properties could give opportunity to obtain materials with a new set of physico-chemical and biological feature. These new materials could find different applications, for example in drug delivery systems or scaffolds for bone and tissue regeneration

Polyvinylpyrrolidone(PVP), also commonly called polyvidone or povidone, is a water-soluble polymer in water and other polar solvents, made from the monomer *N*-vinylpyrrolidone. . When dry it is a light flaky hygroscopic powder, readily absorbing up to 40% of its weight in atmospheric water. In solution, it has excellent wetting properties and readily forms films. This makes it good as a coating or an additive material to coatings.. PVP was initially used as a blood plasma substitute and later in a wide variety of applications in medicine, pharmacy, cosmetics and industrial production. PVP is also used in many technical applications as an adhesive inglue stick and hot-melt adhesives, as an emulsifier and disintegrant for solution polymerization, for production of membranes, such as dialysis and water purification filters(21).

Up to now, there are many different reports for silicone block copolymers in the literature. These include PDMS-*b*-poly (methyl methacrylate), PDMS-*b*-poly (amide-6), PDMS-*b*-poly

(sulfone), PDMS-*b*-poly (bisphenol-A), PDMS-*b*-poly (styrene), PDMS-*b*-poly (ethylene glycol) and PDMS-*b*-poly (urethane) (10-15) and etc. In these early publications, such block copolymers were usually characterized with regard to their surface properties, using microscopic techniques like transmission electron microscopy (TEM) or scanningelectron microscopy (SEM). The recent research in this field is focused on specific applications and often includes cell studies in order to investigate toxicity, cell-uptake, and other biomedical important parameters (16, 17).

In this report, we presentdi-block copolymers consisting of fixed hydrophobic PDMS block chain length and PVP (Poly N-vinyl pyrrolidone) hydrophilic polymer block with different chain lengths. From these poorly studied di-block copolymers we prepared nano-containers (polymersomes) for drug delivery application, accomplished by film rehydration, solvent evaporation and extrusion methods and nano-ranged 3D amphiphilic polymer scaffolds, using versatile technique such as electrospinning. All materials are characterized by different spectroscopic, microscopic technique and are investigated for biological responsein order to find applications in drug delivery and as scaffolds for tissue engineering and regenerative medicine.

Materials and Methods

Synthesis of PDMS-*b*-PVP

The amphiphilic block copolymer with two different PDMS chain lengths was synthesized and characterized in detail in our previous work [9].

Preparation of polymer vesicles

Preparation of polymer vesicles was accomplished by film rehydration, solvent evaporation and extrusion methods For film rehydration method accompanied with sonication and freeze-thaw cycles, 2 ml of 1mg/mL of each block copolymer in tetrahydrofuran was uniformly coated on the inside wall of test tube Duran® with straight rim (Roth, Germany), followed by evaporation of tetrahydrofuran under stream of gaseous nitrogen for 5 min at room temperature and further dried again for 12 h in a vacuum oven at 35^{o}. Extrusion was performed by stainless steel mini extruder (Avanti Polar Lipids, Inc., US) equipped with different pore size polycarbonate membranes and two micro syringes (Hamilton, US). Vesicles suspension was introduced into a 1.0 mL syringe, pushed through 200, 100, 80 and 50 nm membranes, collected into the second syringe and then pushed again in opposite direction.

Electrospinning Set Up

The electrospinning setup by which were prepared 3D fibersis described in details in (17). Briefly, for preparation of the scaffolds are used two block copolymers PDMS4k-*b*-PVP and PDMS10k-*b*- PVP with different molecular weights and they were dissolved in chloroform at various concentrations (10 - 23 wt %), stirred at room temperature for 12 hours. For fiber preparation was used high voltage supply (CZE-30PN0.25, Matsusada Precision Inc., Japan).

SEM observation

Scanning electron microscopy (SEM) Quanta FEG 200 (FEI Europe BV, Branch Belgium) equipped with energy dispersive X-ray (EDX) system for identifying the elemental composition of the specimen, or an area of interest was used. The morphology of the self-assembled block copolymer films was observed at electron acceleration of 7 kV without Au/Pd coating for the samples, and images at various levels of magnification were captured.

Cells

Human fibroblasts were prepared from fresh skin biopsy and used up to the ninth passage. The cells were grown in Dulbecco's minimal essential medium (DMEM) con-taining 10% fetal bovine serum (Sigma) in a humidified incubator with 5% CO2. For the experiments, the cells were harvested from nearly confluent cultures with 0.05% trypsin/0.6 mM EDTA (Sigma).

LDH test

The cell proliferation was determined via modified lactate dehydrogenase (LDH) assay (Hoffman La Roche, Penzberg, Germany) after 1, 3, and 5 days of incubation. Briefly, at the indicated incubation time, the medium was removed and the cells were lysed with 0.5 mL 1% Triton-X 100 in PBS under shaking for 1 h. The cells lysates were centrifuged at 2000 x g for 5 min. Thereafter, 100 µL of LDH test solution was added to each well, and the samples were incubated for 15 min at room temperature in dark. The reaction was stopped with 50 µL 1M HCl. The absorbance was measured with Spectra Flour Pluplate reader (Tecan, Crailsheim, Germany) at 492 nm. The reference wavelength was at 620 nm. Each experiment was quadruplicated.

Actin staining

Overall cell morphology was observed by actin staining. Fibroblasts with density of 1.5 × 105 cells/ml were cultivated on cover glasses (18 × 18 mm), placed in 6-well plates for 24 h. After the incubation period, the non-adhered cells were removed by triple rinsing with PBS (pH 7.4). The adhered cells were fixed with 1 mL 3% solution of paraformaldehyde (PFA) for 15 minutes at room temperature. The fixed cells were permeabilized using 1 mL 0.5% solution of Triton X-100 for 5 minutes and then incubated with 1 mL 1% solution of serum bovine albumin (BSA) for 15 minutes. The samples were washed three times with PBS (pH 7.4) and incubated for 30 min at room temperature with BODIPY 558/568 phalloidin. Again, the samples were washed three times with PBS and once with distilled water, and then were installed on objective glasses by Mowiol. The samples were analyzed using inverted fluorescent microscope (Leica DMI3000 B, Leica Microsystems GmbH, Ger- many) with objective HCX PL FLUOTAR 63x/1.25 oil.

Results and Discussion

We studied the effect of physico-chemical properties of synthesized materials (polymerosomes and 3D fiber scaffolds) on the biological response - cell proliferation, adhesion and morphology.

Producing of polymerosomes

From synthetized block copolymers with fixed PDMS chain length and different PVP chain lengths we have prepared two different types of nano materials – polymersomes and fiber mats. On Figure 1 are presented SEM micrographs of PDMS-*b*-PVP (Fig 1, A) polymersomes.

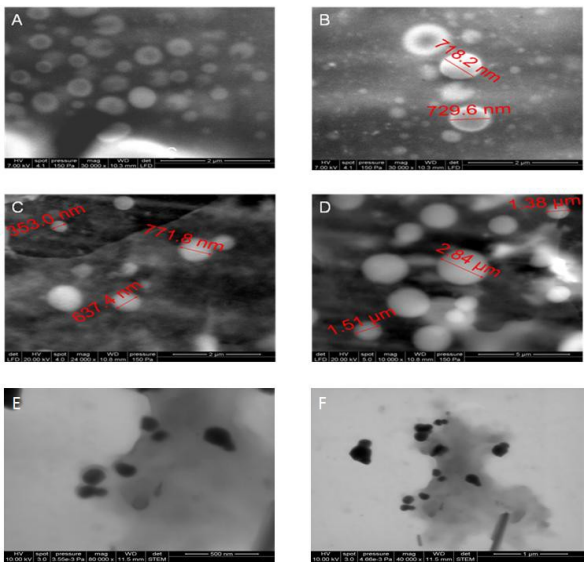

Fig. 1 SEM micrographs of PDMS4k-b-PVP vesicles obtained by film rehydration –A) polymerosomes, B) polymerosomes size700nm, C)300-600nm, D)1.5-3µm, E) and F) polymerosomes after extrusion size100-200nm (16)

In all samples prepared by film rehydration and solvent evaporation methods was observed manypolydispers vesicles and the diameter varied innanometer scale (350-800 nm) (Fig. 1, B and C) to micro meter scale (1.50-3 µm) (Fig. 1, D). Moreover, the indentations in the centre of the vesicles (Fig. 1, A and B) indicate that they are deflated hollow spherical particles. Similar to the conventional phospholipids these vesicles consist from hydrophobic part (PDMS) and hydrophilic shell made by PVP segment. We consider that the large polydispersity may be due to the relatively high polydispersity of the synthesized block copolymers. On the other hand, the differences in diameter in water (~15 µm) and dried state (350-3000 nm) are due to the polymer swelling and diffusion and evaporation of water through the PDMS hydrophobic membrane after drying. A close examination of the contours gives a thickness of the hydrophobic wall (measured in STEM mode)of the polymeric vesicles was measured to be approximately 12.5 nm ±0.3 (drying state, at room temperature), which is higher than this in liposomes as recently reported (20) the vesicles of diblock polymers with a thickness of ca. 8 nm proved to be 5-50 times as tough as the vesicles made of phospholipids and cholesterol

We expect that our spherical membranes have good mechanical property. Initially, the main fraction of PDMS-b-PVP vesicles exhibited size in the range of 500 nm but also were found vesicles with different sizes (Fig 2,A).In order to generate copolymer vesicles with narrow size distribution we have used nano-extruder equipped with polycarbonate membranes with pore size from 50 nm to 200 nm. After extrusion, comparatively small, monodisperse vesicles just over 100 nm and close to 200 nm were generated

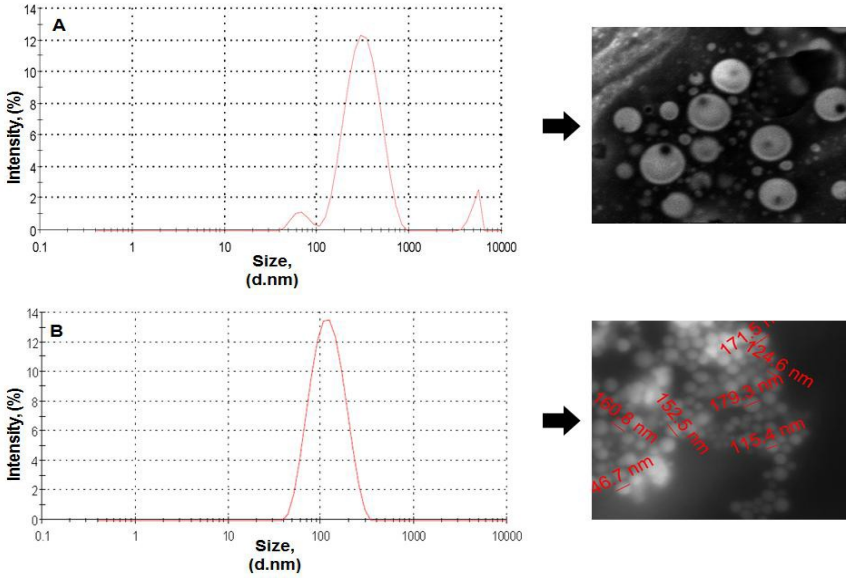

Fig. 2. Average size distribution graphs of PDMS-b-PVP vesicles obtained by film rehydration or solvent evaporation method (A) and (B) vesicles obtained after 50 times extrusion through 100 nm polycarbonate membrane, supported by SEM. (16) as verified by dynamic light scattering (Fig 2, B and D) and SEM images obtained in STEM mode (Figure 1, E and F). Finally, PDMS10k-*b*-PVP and PDMS4k-*b*-PVP with approximately equal PDMS chain, increased the hydrophobic nature of PDMS-*b*-PVP copolymers provided poor solubility in water, and that is why we failed to obtain stable suspension of nanoparticles for increased PVP chain (PDMS4760-*b*-PVP5120 and PDMS10000-*b*-PVP15188).

Preliminary investigations in cell growth and proliferation onto thin layer of the block copolymers show that the samples are with good potential for drug delivery or tissue engineering (16). It is known that the shape and the size of the biomaterial particles as well as surface roughness of thin layers also influence the cell recognition ability and interaction (16). Because of these reasons, we have investigated the influence of our co-polymers on the cell proliferation.

Biocompatibility test

The capability of different PDMS-*b*-PVP samples to support cell growth over a period of 7 days was studied using LDH assay. As illustrated in Figure 3, the PDMS4k-*b*-PVPtends to increase the cell growth on the 3rd and 7th day of incubation. This effect depends maybe onthe hydrophilicity of PVP chain and favorabletopography which support cell proliferation.

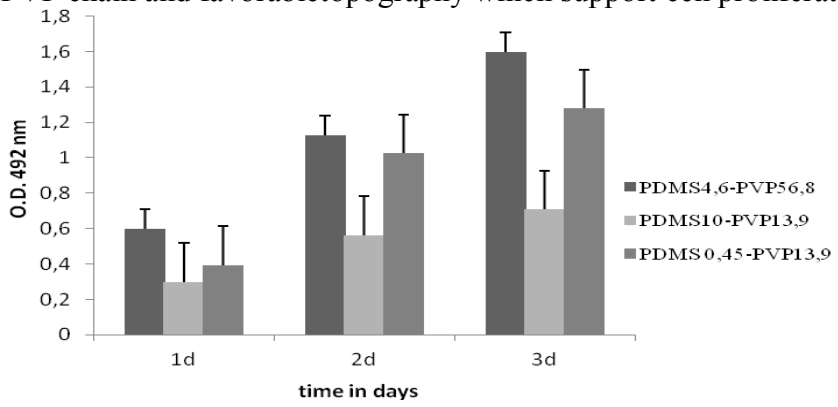

Fig. 3. LDH test for cell viability with human fibroblast seeded onto PDMS- b-PVP polymerosomes (16).

Our preliminary biocompatibility tests showed that the both copolymers: PDMS4k-*b*-PVP and PDMS10k-*b*-PVP are promising materials for biomedical application. The results of the preliminary LDH testindicate better cell viability for the co-polymers PDMS4K-*b*-PVP with longer PVP chain comparing to the copolymer with shorter PVP chains and polymers with PDMS10k-bPVP. This was expected because since PVP is with hydrophilic nature and this copolymer surface is very attractive substrate for the cells growth and proliferation. Additionally, the co-polymer surfaces probably possess different surface roughness and this makes the co-poplymer with longer PVP chain length a preferable surface for cell growth. Finally, we believe that these polymer nanocapsules possess great potential for encapsulation and controlled release of “guest”molecules in/from their interior, especially since the controlled formation of these structures can be achieved rather easily and the composition of the underlying diblock copolymer. This would allow adapting, for example, the permeability of the nanocapsules to the desired application.

Producing of 3D fiber scaffolds

From PDMS-b-PVP block co-polymers and electrospining technique was obtained 3D fiber scaffold.

The electrospinning technique was employed in order to fabricate polymer nanofiber mats from PDMS-*b*-PVPblock copolymer. We varied the applied voltage from 6 to 30 kV and the flow rate was between 10 and 30 μL/min. The distance between tip and collector was fixed to 14 cm. In the copolymer, comparing to PDMS-b-PVP copolymer where PVP block is several times longer than PDMS block (Fig. 4).

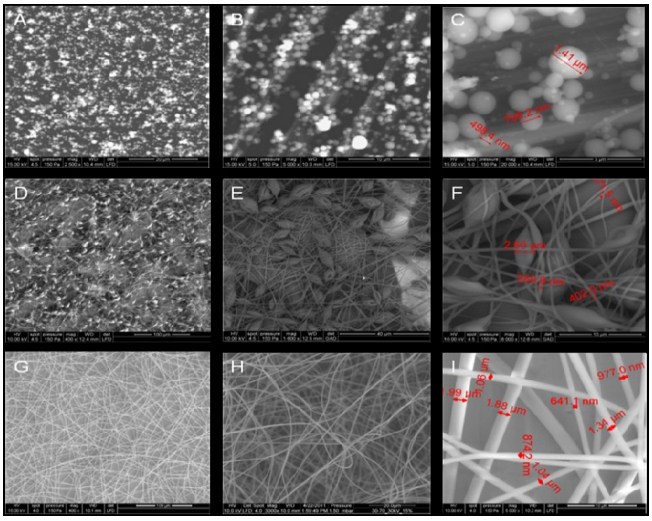

Figure 4. SEM images at different magnification (A), (B) and (C), from 3 μm to 20 μm for spherical particles and (D)-(I), from 10 μm to 100 μm for fibers) of PDMS-*b*-PVP electrospun fibers from chloroform solutions with different concentrations 0 wt% (A), (B) and (C), 15 wt% (D), (E) and (F) and 18 wt% (G), (H) and (I).(17)

Water vapor uptake studies were performed on the electrospun fibers with different PVP content in order to evaluate hydrophilicity and the changes in morphology at room temperature and constant humidity(17).With increasing of PVP content the hydrophilicity increases and for samples with the highest PVP content (PDMS10K-*b*-PVP21.4K and PDMS4.6K- *b*-PVP177.5K) appeared very hydrophilic. It is of interest to note that the water absorption equilibrium was reached after approximately 8 days for both copolymer fiber mats. The surface of block copolymer fibers is highly hydrophilic in the case of electrospun fibers from copolymer with short PDMS chain, reporting contact angles between 36° for PDMS4.6K-*b*-PVP0.45K and 24° for PDMS-*b*-PVP Water spread out immediately upon contact with the surface of fiber mats (16).

It must be noted also that the fibers morphology changes completely after reaching the swelling equilibrium from fibers to polymer gel (Figure 5). This is because PVP is water-soluble polymer and PDMS do not prevent the inhomogeneous fiber mats from complete dissolving/swelling process, resulting at the end in lack of fibrous structure.PVP generates a highly hydrophilic fiber mat and this could be advantage in tissue engineering or may have potential application in drug release systems.

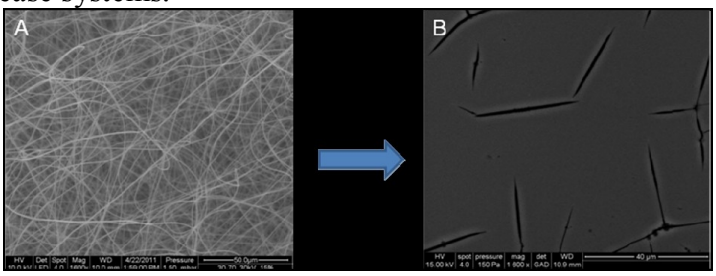

Fig.5Effect of water adsorbtion on the morphology of electrospunPDMS-b_PVPuniform fibers obtained with 16 kV,total polymer concentration18 wt % A)before water adsorbtion B) after24h water adsorbtion(17).

Biocompatibility test

In order to understand the degree of biocompatibility of our copolymer nanofibers we have studied how they can support fibroblast growth over a period of 7 days.

The cell viability was shown todepend on the differenthydrophilic/hydrophobic sectorsin the electrospunned copolymer scaffolds as well as fiber roughnesswith beads in the fibers morphology

or uniform fibers.From the literature it is known that PVP in high concentration hasa cell repellenteffect on the polymer surface (17).The cell adhesion is well expressed on the 3D electrospunned scaffold. The biological investigation onto PDMS-*b*-PVP fiber scaffold revealed that the scaffold has good biocompatibility, but lower cell proliferation.

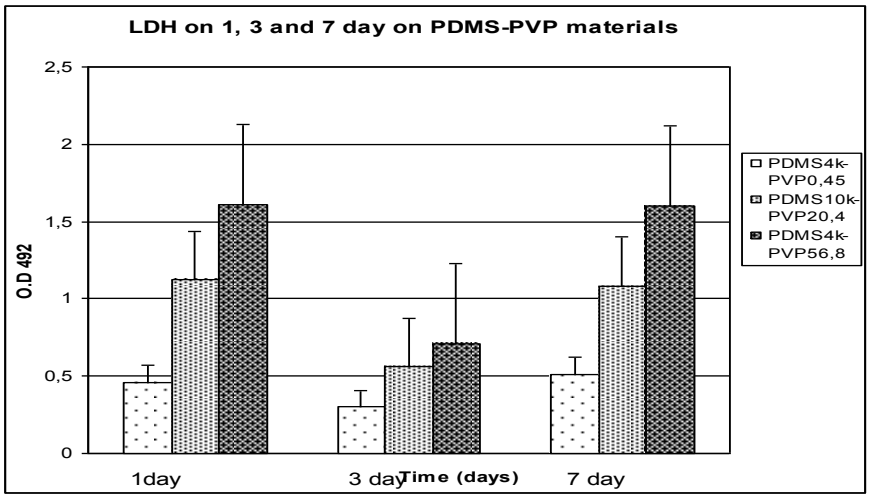

Fig 6 Cell proliferation of fibroblast cells on 1, 3 and 7day on $PDMS_{4,6K}$-*b*-$PVP_{56,8K}$ and $PDMS_{4,6K}$-*b*-$PVP_{0,45K}$, $PDMS_{10K}$-b-$PVP_{13,9K}$(17)

LDH method shows that the cells grow on 1, 3 and 7 day on PDMS4K-*b*- PVP and less on PDMS10K- *b*-PVP. Significant statistical changes we found between the first and third days of cell proliferation. The cell proliferation confirms our morphological investigations.

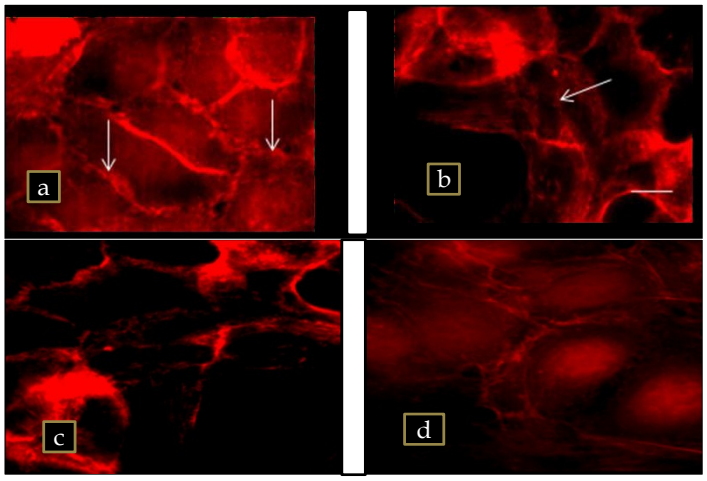

Figure 4 Fluorescent microscopic images of actin staining of fibroblasts seeded on different PDMS-b-PVP surfaces with nano-containers: (a) PDMS4K-b-PVP0, (b) PDMS10K-b-PVP and electrospun scaffolds:(c) PDMS4K-b-PVP (d) PDMS10K-b-PVP1.Bar 100µm (16, 17).

On Figure 4 are presented fluorescent microscopic images of actin staining of fibroblasts seeded on PDMS-b-PVP materials. It can be seen that the cell adhesion isweak on the surfaces. Actinwas diffusely distributed near to the cell border (showed with white arrows)(Fig4, a- d)which

is a sign for week cell adhesion.We concluded that in general PDMS-b-PVP polymerosomes and fiber mats do not support cell adhesion.

Conclusion

In this work we present two diblock copolymers PDMS-b-PVP from which we have prepared polymersomes and 3D fiber mats. It must be not that for the first time we have investigated the self-assembling properties in water and the preparation of nano-ranged amphiphilic copolymer fibers. We consider that this promising material, consisting of bioinert PDMS block and well known biocompatible and hydrophilic PVP block, possessing very interesting properties such as good biocompatibility and amphiphilicity reported here, and could find application and drug delivery systems We found that both copolymers promote cell proliferation as for both type of materials (polymersomes and fiber mats) the better materials for cell growth was that containing longer PVP chain. But both polymers organized in polymerosomes and nanofibers do not support well cell adhesion most probably because of the changes occurring upon the swelling equilibrium of the polymers (17) and unfavorable spatial organization of di-block polymers. Nevertheless, the structure of polymersomes with highly hydrophobic core consisting of PDMS block and the hydrophilic shell consisting of PVP polymer chain could find application in drug delivery systemsbecause of biocompatibility of this new surface and long life of polymerosomes of PDMS-b-PVP block copolymer.

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