Molecular and Cell Biotechnologies

Copolymeric Hydrogel Membranes For Immobilization And Cultivation Of Human Stem Cells

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Significant amount of hydrogel membranes for stem cells cultivation has been synthesized on the basis of acrylamide, acrylonitrile, and acrylic acid by the method of radical copolymerization. The comparative investigations have shown that the optimal parameters, for the immobilization of human mesenchymal stem cells are inherent to nonionic copolymer hydrogels on acrylamide and acrylonitrile bases. The use of hydrogels with considerable acrylic acid content is limited by pH decrease in cultivation medium, which is incompatible with life activity of the cells under investigation.

Key words: hydrogel, stem cells, immobilization, cultivation medium.

Introduction. The development of biotechnologies based on cell preparations in transplantology requires the use of biocompatible polymeric matrixes and the carriers suitable for immobilization and cultivation of stem cells and specialized cells *in vitro* [1].

Copolymeric hydrogels – cross-linked hydrophilic polymers with three dimensional structure – find a wider application for medicine and industry in the recent years. Depending on chemical nature of monomers, used for the

synthesis (first of all, hydrophily, polarity and the presence of ionogenic groups), physico-chemical parameters of the obtained hydrogels may be changed purposefully in the wide range. On the basis of different hydrogels the following soft lenses were obtained with the improved supply of eye cornea with oxygen; antiglaucoma ophthalmic films with the prolonged hypotensive substances liberation, dense nutrition medium for cultivation of microorganisms, antiburn material, which combines effective exudation absorption with prolonged liberation of a wide specter of chemotherapeutical agents etc [2-6].

Polymeric hydrogels are used for immobilization of microorganisms and cells. It was demonstrated that the ethylene production effectiveness was 3.5 times higher by the yeast cells immobilized on the hydrogel carrier in comparison with free cells [7]. The composite material based on copolymeric hydrogel and cells precursor are used for the formation of new bone and cartilaginous tissues in rehabilitative and reconstructive surgery [8]. Acrylic latex with immobilized *Escherichia coli* cells is suggested to be used as biocatalyst [9].

Polyacrylamide gel is characterized by high hydrophility and biological tolerance, which, along with other operational characteristics, makes it suitable for immobilization of cells and microorganisms. At the same time it has some disadvantages, among which insufficient mechanical strength and the absence of ionic groups that could assist the immobilization of cells on its surface. In order to find the optimal hydrogel matrix content for cells cultivation a great number of copolymeric hydrogels based on acrylic monomers were synthesized.

Materials and Methods. The hydrogels were obtained in water medium at room temperature by the method of radical copolymerization. The process of gel formation was induced using potassium persulfate - natrium metabisulphate oxidation-reduction system. All together 17 hydrogels of different content were synthesized. Monomer ratio was varied – acrylamide, acrylonitrile, and acrylic acid in the range from homopolyacrylamide gel to copolymeric hydrogels with 50% copolymer content. Total monomer content in hydrogel was from 20 to 50%. Cross-linking with spatial frame was provided using bifunctional monomer – N,N -methylene-bis-acrylamide. Cross-linking agent concentration was varied in the range from 0.188 to 0.75%.

The hydrogel samples of the investigated composition were poured in special templates, exposed for 1 hour and then parted. The samples were immerged into significant amount of distilled water in order to wash off the mixture components, which did not react and exposed for 10 days at 651C.

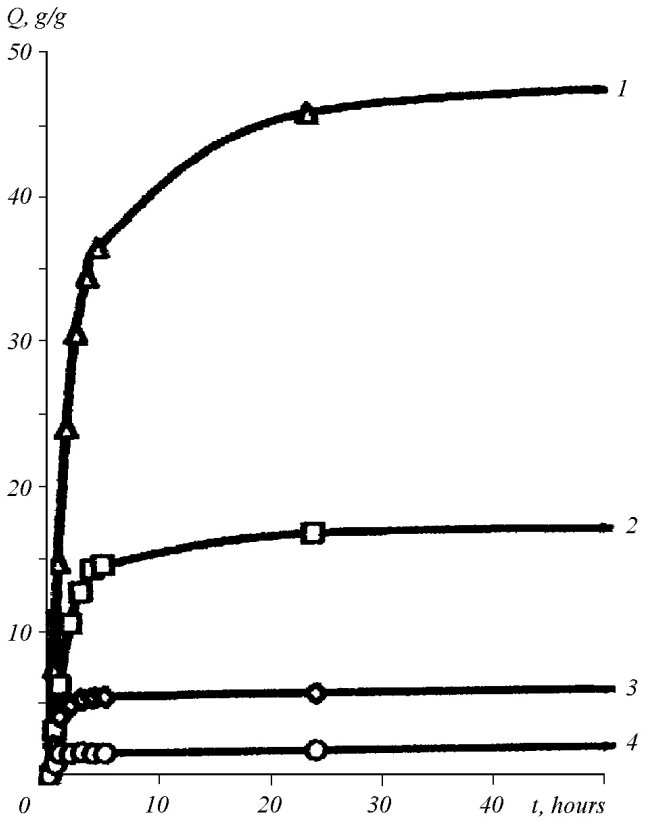

Fig.1. The kinetics of hydrogels swelling of different content in distilled water at 201C: 1 – hydrogel copolymer of acrylamide and acrylic acid (AC); 2 – polyacrylamide based hydrogel; 3 – hydrogel copolymer of AC and acrylonitrile (AN); 4 – hydrogel copolymer of AA and AN.

To test hydrogel membranes (as investigated cell line carriers) Petri dish surface was covered by membrane samples and poured over by the cultivation medium for not less than 5-6 hours for hydrogel swelling. Medium surplus was removed and cell suspension was applied (10^5 cells into 2 ml of cultivation medium). Cell suspension was prepared on the basis of mesenchymal stem cells of B4L2 line, obtained previously from peripheric donor blood. The monolayer of cells was treated by tripsin and versen solutions. The standard method of cells cultivation is described in [10]. B4L2 cells were cultivated *in vitro* in standard medium DMEM (Sigma, USA) adding 5% of new born calf serum (Sigma) and antibiotics of penicillin and streptomycin (100 units/ml each). The cells were cultivated with the monolayer formation on the surface of glass vial or Petri dish (Anumbra), d=35mm.

The cells were sown on glass surface of standard Petri dishes. Further observations of cells were performed using inverted microscope every day for 1 week. During this time the cells capability to immobilization on the investigated

Calculation parameters of hydrogel mathematic model

Gel No.	Qmax	a	1/	t0
1	47.5	0.68	0.234	1
2	16.5	0.58	0.434	1
3	5.5	0.38	0.984	1
4	1.5	0.99	4.0	0

Fig.2. General appearance of hydrogel membranes after swelling in cultivation medium DMEM.

hydrogel membranes, adhesive properties, the abilities for growth, reproduction, and morphology were evaluated.

Results and discussion. One of the fundamental features of hydrogels is the sorption capability to water solutions, which determines their saturation by nutrition medium for cells and microorganisms cultivation.

The swelling of samples Q was calculated according to the formula

$$Q \quad \frac{m_2 \quad m_1}{m_1}, \qquad (1)$$

m_1 – dry sample weight, m_2 – swollen sample weight

The kinetics of synthesized hydrogel swelling in distilled water is shown in Fig.1. The experimental data are represented by marker-dots. As it is seen from the data presented, kinetic dependencies look like saturation curve and may be represented by the mathematic model

$$Q \quad Q_{\max}(T,C,G,M) \ (1 \quad a \ \exp(\ \frac{t \quad t_0}{}), \qquad (2)$$

Q_{max} – maximum possible swelling at current conditions in certain medium determined by the gel content and properties; T, C, G, M – generalised marking for conditions parameters, gel content etc; a, t_0, ф – the parameters, which characterise the saturation process; t - time.

The deviation between the experimental data and theoretically (using the mentioned formula) calculated one was determined by the smallest square method. Its value did not exceed 5% of the maximum value. Equation (2) coefficients are presented in the Table.

It is evident that gel 1 is capable of adsorbing the solvent (Q_{max}) more, but with the lower adsorption speed (1/ф), at the same time gel 4 adsorbs less liquid but with the higher speed. The major part of solvent, in which the hydrogel swelling takes place, is sorbed during 5-6 hours.

Q_{max} swelling decreases at the increase of cross-linking agent concentration. Replacement of acrylamide links for acrylonitrile links in polyacrylamide gel also decreases hydrogel swelling. The dependence of swelling on acrylic acid links content has a more complicated character, moreover maximal swelling corresponds to 25% acrylic acid hydrogel.

The mechanical strength of hydrogels increases in accordance to the replacement of acrilamide links for acrylic acid links in polyacrilamide gel and especially for hydrophobic acrylonitrile links. However, regardless of the improvement of mechanical characteristics of copolymeric hydrogels with high acrylonitrile content, due to high heterogeneity they lose transparency, which makes it difficult to use them for cultivation and to control the growth of cells and microorganisms on them. The hydrogels with

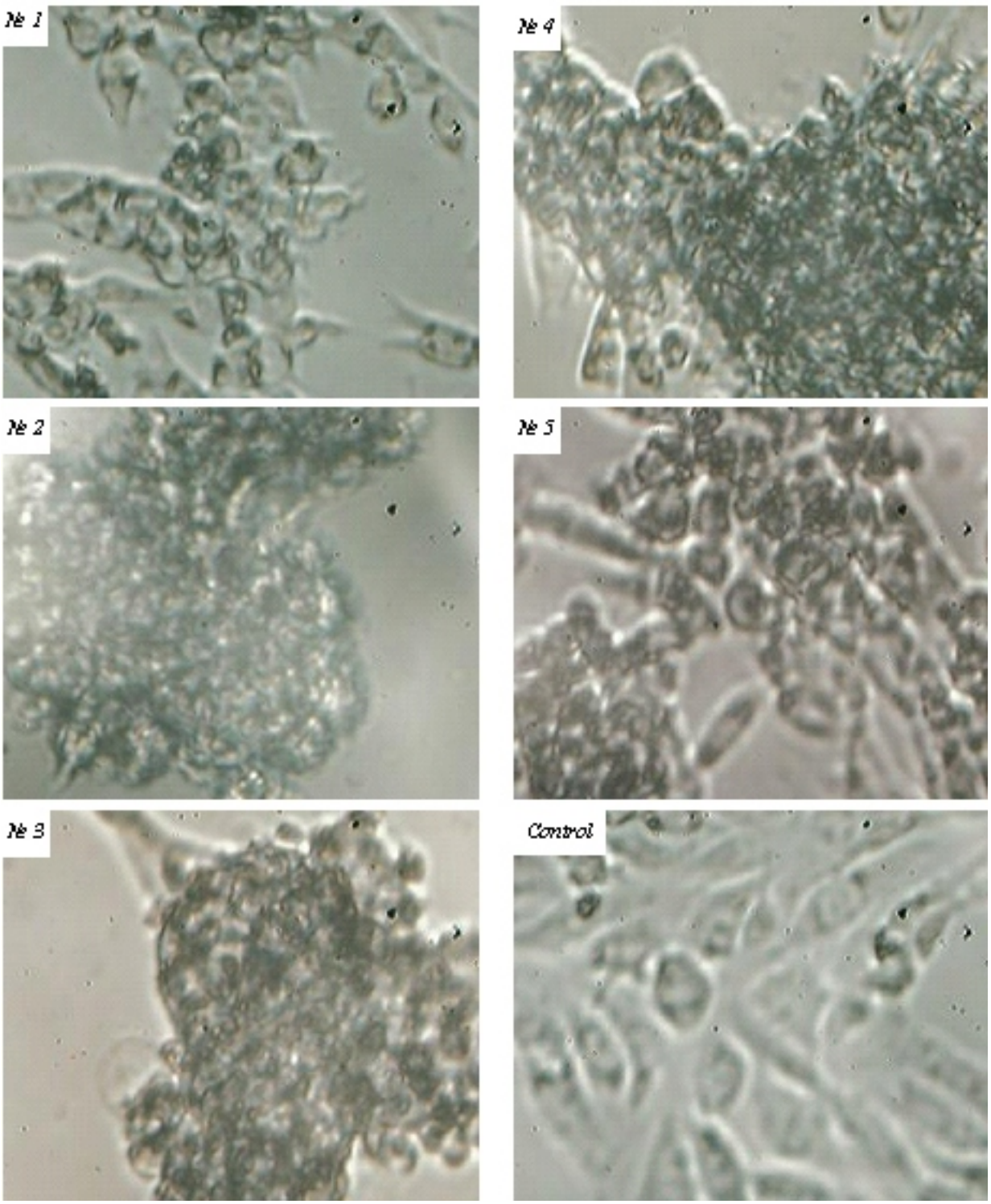

Fig.3. The immobilization of cells and the formation of conglomerates on hydrogel membranes (No.1-5) and glass surface of vial in control.

sigFig.3. The immobilization of cells and the formation of conglomerates on hydrogel membranes (No.1-5) and glass surface of vial in control.nificant acrylic acid content turned out to be unusable for life activity maintenance of cells due to acid reaction specific to them, which led to abrupt pH change of cultivation medium.

General appearance of hydrogel membranes after their swelling in cultivation medium DMEM is shown in Fig.2.

In order to determine cells compatibility with the obtained hydrogel membranes, the attaching and spreading of cells on new substrates were investigated using inverted microscope. The cells growth and reproducibility at these conditions were determined after their cultivation *in vitro* for several days. In one day after sowing cells onto acrylamide based hydrogel membranes, the major part of samples showed the attaching of the cells to their surface, partial spreading, as well as the aggregation of cells into conglomerates of different size with further releasing from substrate and transforming into suspension state (Fig.3, No.1, 3-5). The strength of attaching was tested by mechanical stirring the membranes together with the immobilized cells in the medium. The most effective cell spreading in the process of cultivation was observed for the membranes which contain the biggest amount of cross-linked polymer in gel and maximal cross-linking frequency (Fig.3, No.2). The picture of cells spreading on the substrate in this variant is similar to monolayered cells on the glass surface of standard vials in control (Fig.3, control).

The characteristics of intercellular interactions and the interaction of cells with the substratum at cells immobilization on the hydrogel membranes are the important qualitative mark of investigated objects biocompatibility. If hydrogel membrane content is not compatible with the cell adhesive properties, the cells try to avoid the contact with the base, first of all, to decrease the contact area, which leads to the cell aggregates formation. The cell aggregates depart from the membrane and turn to suspension while adhesive force decreases. And *vice versa* when hydrogel content is compatible with cell adhesion properties appearance, they spread on substrate and multiply, like the control samples.

Conclusions. Therefore, the copolymeric hydrogels based on acryl row monomers may be characterized by the set of parameters, the determination of which simplifies the process of their obtaining and settling compatibility with immobilized cells. Hydrogel membranes may be used not only for immobilization of microorganisms, but also for the cells of higher eukaryotes, which has been shown by us on the example of human stem cells. It is possible that at certain modification of hydrogel structures, they may be stored for a long time under cryopreservation.

The estimation of cultivated cells state allowed defining that the optimal operational parameters, from the point of view of life activity maintenance, immobilization and adhesion of cells on membranes surface, are specific to hydrogels based on high concentration acrylamide. The usage of hydrogels with significant content of acrylic acid is limited by decreasing of pH cultivation medium, which is incompatible with the vitability of the investigated cells. In the next article there will be a new data considering the technological conditions, which are necessary for effective and long-lasting cells cultivation on the hydrogel membranes surface with the improved operational properties.

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Кополимерные гидрогелевые мембраны для иммобилизации и культивирования стволовых клеток человека

Резюме

На основе акриламида, акрилонитрила и акриловой кислоты методом радикальной кополимеризации синтезированы гидрогелевые мембраны для культивирования стволовых клеток. В результате сравнительных исследований установлено, что оптимальные параметры, определенные иммобилизацией мезенхимальных стоволовых клеток человека, присущи неионогенным кополимерным гидрогелям на основе акриламида и акрилонитрила. Использование гидрогелей со значительным содержанием акриловой кислоты ограничено снижением величины pH культуральной среды, что несовместимо с поддержанием жизнедеятельности исследуемых клеток.

Ключевые слова: гидрогель, стволовые клетки, иммобилизация, питательная среда.

REFERENCES

1. Лукаш *Л. Л., Василовская С. В.* Стволовые клетки млекопитающих *in vitro* как основа для создания современных биотехнологий // Биополимеры и клетка.–2001.–**17**, № 3.–С. 203–211.
2. *Burczak K., Camian E., Kochman A.* Long-term in vivo performance and biocompatibility of poly (vinyl alcohol) hydrogel macrocapsules for hybrid-type artificial pancreas // Biomaterials.–1996.–**17**.–P. 2351–2356.
3. *Rosiak J., Burczak K., Pekala W.* Polyacrylamide hydrogels as sustained release drug delivery dressing materials // Radiat. Phys. Chem.–1983.–**22**.–P. 907–915.
4. *Филиппова О. Е.* «Восприимчивые» полимерные гели // Высокомолекуляр. соединения.–2000.–**42**, № 12.–С. 2328–2352.
5. *Chen J., Park K.* Superporous hydrogels: fast responsive hydrogel systems // J. Macromol. Sci.-Pure and Appl. Chem.–1999.–**36**.–P. 917–930.
6. *Самченко Ю. М., Ульберг З. Р., Комарський С. А.* Кополімерні гідрогелі медичного призначення – наукові основи розробки лікарських препаратів.–Харків: Основа, 1998.–С. 159–177.
7. *Lu Zhaoxin, Fujimura T.* Immobilization of yeast cells with ionic hydrogel carriers by adhesion- multiplication // J. Agr. and Food Chem.–2000.–**48**.–P. 5929–5932.

8. *Pat.* USA N 6171610. Guided development and support of gydrogel-cell compositions / C. Vacanti, J. Vacanti, M. Vacanti // Publ.
9. *Venkata S. Thiagarajan, Zhisong Huang, L. E. Scriven, Janet L. Schottel, Michael C. Flickinger.* Microstructure of a biocatalytic latex coating containing viable Escherichia coli cells // J. Colloid and Interface Sci.–1999.–N 215–P. 244–257.
10. *Бужиевская Т. И., Лукаш Л. Л., Подольская С. В.* Экспериментальные модели для изучения мутагенеза и трансформации, индуцированных вирусами и нуклеиновыми кислотами // Методы молекуляр. биологии.–Киев: Наук. думка, 1986.–С. 147–158.

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