Annex no. 2

SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

1. NAME AND SURNAME: Alicja UTRATA-WESOŁEK

2. DIPLOMAS AND SCIENTIFIC DEGREES:

June 2001	**Master of Science,** specialization Chemical Technology and Polymer Chemistry Faculty of Mathematics, Physics and Chemistry, Basic and Applied Chemistry, University of Opole
	diploma thesis granted by PTChem "Janina Janikowa" award:
	„Copolymerization of ethylene with 1-hexene using zirconocene catalyst supported on $MgCl_2(THF)_2$"
	Supervisor: Prof. dr hab. Krystyna Czaja
July 2006	**Doctor of Chemical Sciences**
	Silesian University of Technology in Gliwice, Poland Faculty of Chemistry
	Ph.D. thesis: "Stimuli sensitive polymers based upon reactive polyethers", thesis granted by the faculty the degree of excellency "summa cum laude"
	Supervisors: Prof. dr hab. Andrzej Dworak; Prof. dr hab. Brigitte Voit

3. COURSE OF THE EMPLOYMENT – INFORMATION ABOUT PAST AND CURRENT EMPLOYMENTS IN SCIENTIFIC UNITS

Positions:

november 2001 – october 2005	**Ph.D. student** at the Faculty of Chemistry, Silesian University of Technology
november 2005 – july 2007	**specialist** in Institute of Coal Chemistry Polish Academy of Sciences
july 2007 – march 2017	**research scientist (adiunkt)** in Centre of Polymer and Carbon Materials Polish Academy of Sciences in Laboratory of Nano- and Microstructural Materials
march 2017 to date	**assistant** in Centre of Polymer and Carbon Materials Polish Academy of Sciences in Laboratory of Nano- and Microstructural Materials

Functions:

2011-2014	Member of the Scientific Council of the Centre of Polymer and Carbon Materials Polish Academy of Sciences

4. PRESENTATION OF THR SCIENTIFIC ACHIEVEMENT

resulting from the article 16.2 of the act on scientific degrees and scientific titles and degrees and title in the field of art (Act no. 65, item 595 as amended)

(a) TITLE OF SCIENTIFIC ACHIEVEMENT

The scientific achievement is the series of monothematic articles entitled:
„BIOCOMPATIBLE POLYMER LAYERS OF CONTROLLED AFFINITY FOR WATER. SYNTHESIS AND APPLICATION"

(b) List of selected publications constituting the scientific achievement in chronological order, with percentage contribution of habilitant to each work

Impact Factor (IF) – appropriate to the year of publication

[H1]	Biocompatible cryogels of thermosensitive polyglycidol derivatives with ultra-rapid swelling properties P. Petrov, **A. Utrata-Wesołek**, B. Trzebicka, Ch. B. Tsvetanov, A. Dworak, J. Anioł, A. Sieroń European Polymer Journal **2011**, 47, 981-988	IF=2.739 (contribution = 45%)
[H2]	Photodegradation of polyglycidol in aqueous solutions exposed to UV irradiation **A. Utrata-Wesołek,** R. Trzcińska, K. Galbas, B. Trzebicka, A. Dworak Polymer Degradation and Stability **2011**, 96, 907-918	IF=2.769 (contribution = 60 %)
[H3]	Antifouling surfaces in medical application **A. Utrata-Wesołek**[*] Polimery **2013**, 58, 685-695	IF=0.617 (contribution = 100 %)
[H4]	Poly[tri(ethylene glycol) ethyl ether methacrylate] - coated surfaces for controlled fibroblasts culturing A. Dworak, **A. Utrata-Wesołek**, D. Szweda, A. Kowalczuk, B. Trzebicka, J. Anioł, A. L. Sieroń, A. Klama-Baryła, M. Kawecki ACS Applied Materials & Interfaces **2013**, 5, 2197-2207	IF=5.900 (contribution = 40 %)
[H5]	Modified polyglycidol based nanolayers of switchable philicity and their interactions with skin cells **A. Utrata-Wesołek,** N. Oleszko, B. Trzebicka, J. Anioł, M. Zagdańska, M. Lesiak, A. Sieroń, A. Dworak European Polymer Journal **2013**, 49, 106-117	IF=3.242 (contribution = 60 %)
[H6]	(Co)polymers of oligo(ethylene glycol) methacrylates – temperature-induced aggregation in aqueous solution B. Trzebicka, D. Szweda, S. Rangelov, A. Kowalczuk, B. Mendrek, **A. Utrata-Wesołek**, A. Dworak Journal of Polymer Science Part A-Polymer Chemistry **2013**, 51, 614-623	IF=3.245 (contribution = 10 %)
[H7]	Poly(2-substituted-2-oxazoline) surfaces for dermal fibroblasts adhesion and detachment A. Dworak, **A. Utrata-Wesołek**, N. Oleszko, W. Wałach, B. Trzebicka, J. Anioł, A. L. Sieroń, A. Klama-Baryła, M. Kawecki Journal of Materials Science-Materials in Medicine **2014**, 25, 1149-1163	IF=2.587 (contribution = 50 %)
[H8]	Controlling the crystallinity of thermoresponsive poly(2-oxazoline)-based nanolayers to cell adhesion and detachment N. Oleszko, W. Wałach, **A. Utrata-Wesolek**, A. Kowalczuk, B. Trzebicka, A. Klama-Baryła, D. Hoff-Lenczewska, M. Kawecki, M. Lesiak, A. L. Sieron, A. Dworak Biomacromolecules **2015**, 16, 2805-2813	IF=5.583 (contribution = 40 %)

[H9]	Crystallization of Poly(2-isopropyl-2-oxazoline) in Organic Solutions N. Oleszko, **A. Utrata-Wesołek**, W. Wałach, M. Libera, A. Hercog, U. Szeluga, M. Domański, B. Trzebicka, A. Dworak Macromolecules **2015**, 48, 1852-1859	IF=5.554 (contribution = 20 %)
[H10]	Multiple and terminal grafting of linear polyglycidol for surfaces of reduced protein adsorption **A. Utrata-Wesołek*,** W. Wałach, J. Anioł, A. L. Sieroń, A. Dworak Polymer **2016**, 97, 44-54	IF=3.586 (contribution = 80 %)
[H11]	Transfer of fibroblast sheets cultured on thermoresponsive dishes with membranes M. Kawecki, M. Kraut, A. Klama-Baryła, W. Łabuś, D. Kitala, M. Nowak, J. Glik, A. L. Sieroń, **A. Utrata-Wesołek**, B. Trzebicka, A. Dworak, D. Szweda Journal of Materials Science: Materials in Medicine **2016**, 27, 111	IF=2.272 (contribution = 5 %)
[H12]	Photocrosslinking of polyglycidol and its derivative – route to thermoresponsive hydrogels **A. Utrata-Wesołek***, I. Żymełka-Miara, A. Kowalczuk, B. Trzebicka, A. Dworak Photochemistry and Photobiology DOI: 10.1111/php.12819	IF= 2.121 (contribution = 70 %)

Total Impact Factor of 12 publications constituting the scientific achievement is 40.215

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(c) OVERVIEW OF THE SCIENTIFIC AIMS AND OBTAINED RESULTS PRESENTED IN THE SERIES OF PUBLICATIONS „BIOCOMPATIBLE POLYMER LAYERS OF CONTROLLED AFFINITY FOR WATER. SYNTHESIS AND APPLICATION"

1. INTRODUCTION

Materials made of metals, ceramics, carbon materials and polymers (both natural and synthetic) are often used in modern medicine for the treatment of various diseases [1, 2]. Coronary stents, vascular, knee or hip prostheses, artificial heart valves, various implants or contact lenses are products derived from such biomaterials and play a significant role in improving the health and quality of patients life. In recent years, significant advances have also been achieved in using a biomaterials for targeted drug transport, gene therapy, reconstructive medicine, or tissue engineering.

Biomedical materials that come into contact with body fluids or tissues must possess a specific properties that are adapted to the particular application. Thus it is essential to understand the interaction of biomaterial with biologically active substances such as proteins or cells. For these interactions the surface of biomaterial is responsible [3]. Therefore, the ability to control the biomaterials surface properties, e.g. by coating them with layer made of polymer, is of particular importance for the applications in medicine.

The main research problem that forms the basis of the habilitation procedure, to which this series of publications is dedicated, is the development and characterization of new, biocompatible polymer layers with properties that allow to use the obtained biomaterial in reconstructive medicine as coatings reducing the protein adsorption or in tissue engineering for culture and detachment of cell sheets. To achieve this, the interactions of proteins and cells with polymer coatings should be determined.

The works with the use of synthetic materials for biomedical applications are intensive. Synthesis of polymer layers (e.g. of a linear structure in the form of a polymer brush or crosslinked, dendritic, or layer-on-layer coatings) are described [3, 4]. Unfortunately, based on the presented results, the correlation between the properties of polymer layers resisting protein adsorption or favoring cell culture with macromolecule structure or topology, surface coating method or polymer layer thickness is not known or not sufficiently clarified.

The aim of this work was to develop and compare new biocompatible polymer layers with different composition and structure, and to determine the relationship between the properties of the surface covered with polymers and their interaction with proteins or cells.

In order to obtain the polymer layers reducing the protein adsorption a biocompatible, hydrophilic polymers of glycidol were used. In the laboratory of Nano- and Microstructural Materials CMPW PAN, where the research was conducted, methods of controlled synthesis of polyglycidol of different macromolecular architecture [5,6] were developed. Based on this knowledge and on the fact that there was no data on the use of polyglycidol, except for dendritic one, as anti-protein coatings [7-9], **the studies to obtain coatings made of linear polyglycidol and its copolymers with ethylene glycol and to determine their interaction with proteins were undertaken.**

The biocompatible polymer layers have also been tested for their use as a cell culture surfaces. For this purpose a thermoresponsive polymers were used. The control of the affinity for water (hydrophilic-hydrophobic balance) of the layers made of these polymers, only by changing the envi-

ronmental temperature, is possible. This property allow the thermoresponsive layers to be used in regenerative medicine for culture and detachment of cells in the form of a sheet. As a result of our previous work, a method for modifying the hydrophilic polyglycidol to thermoresponsive polymers has been developed [10]. **Based on this knowledge, a research to obtain thermoresponsive coatings based on modified polyglycidol and the estimation of their interaction with cells has been undertaken. Other thermoresponsive polymers have also been used in this work: poly[oligo(ethylene glycol) methacrylates] and poly(2-substituted-2-oxazoline)s,** as poly(*N*-isopropylacrylamide) (PNIPAM) or its copolymers used to this point for cell sheet culture have some disadvantages (e.g. lack of functional groups available for modification, phase hysteresis or aggregation) [11, 12].

To synthesis the polymers, living and controlled anionic and cationic polymerization as well as atom transfer radical polymerization (ATRP) were applied. These methods allow for precise control of the polymer layer structure, which further ensures the control of properties of the resultant materials under given conditions.

In this work polymer coatings were obtained in a form of self-supporting layers (polymer gels) and as layers immobilized on a support. To bound the polymer with a support grafting-to and grafting-from techniques were applied (Fig. 1.1). Both methods provide covalent bonding of the polymer with the support thus a stable layer can be obtained.

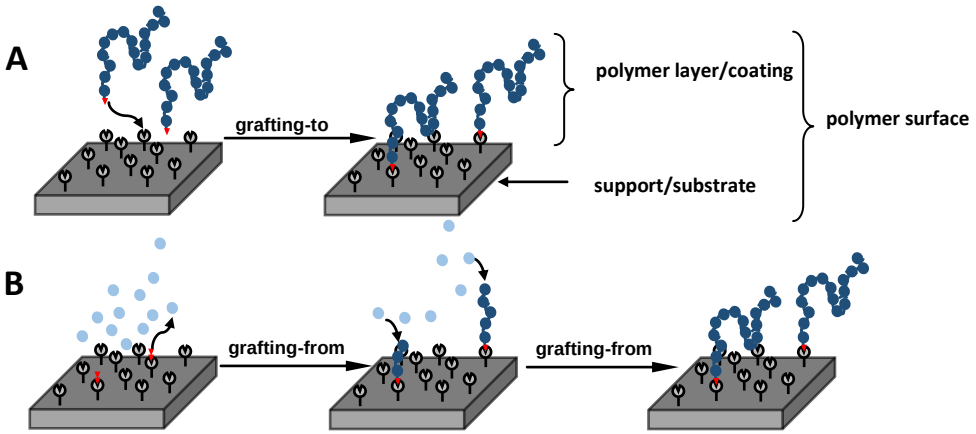
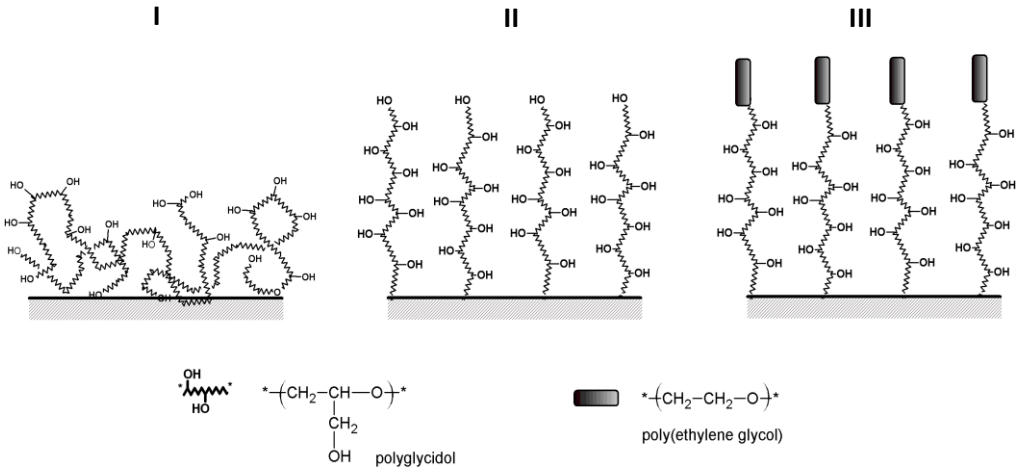


Fig. 1.1. Scheme of covalent bonding of polymer with a solid support using (A) grafting-to and (B) grafting-from techniques.

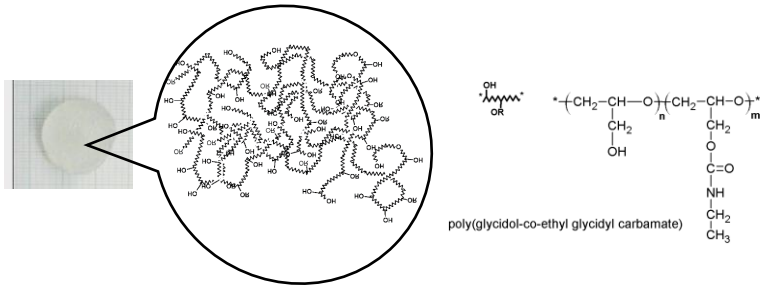
The structure of all polymer layers tested in the habilitation work are shown in figure 1.2. The ability to interact of hydrophilic or thermoresponsive polymer layers with proteins or cells was investigated for layers of different chemical structure and topology.

HYDROPHILIC POLYMER LAYERS REDUCING PROTEIN ADSORPTION



THERMORESPONSIVE POLYMER LAYERS FOR CELL SHEET CULTURE

A. Self-supporting polymer layers



B. Polymer layers immobilized on support

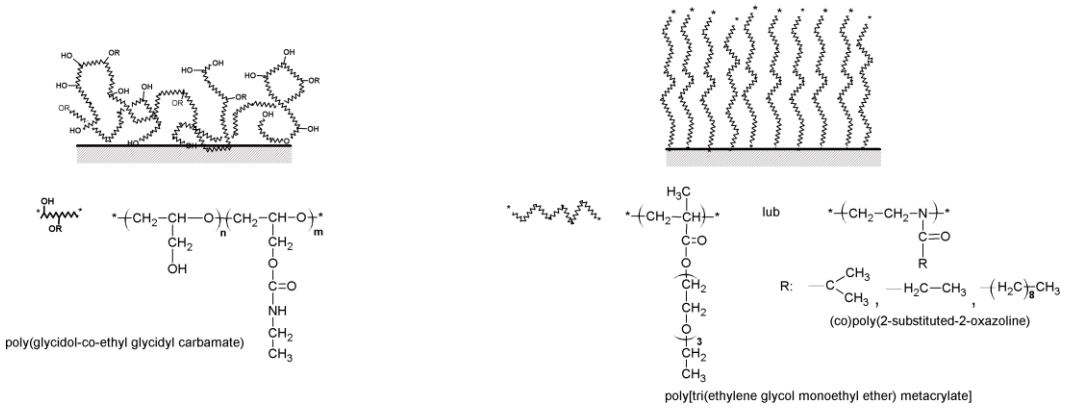


Fig. 1.2. Polymer layers which are the subject of habilitation work.

The habilitation dissertation describes the author's work including:

1. Synthesis and characterization of hydrophilic layers based on polyglycidol **[H3, H10]**
2. An estimation of the influence of polyglycidol structure and polyglycidol layers properties on the reduction of protein adsorption **[H10]**
3. Synthesis and characterization of thermoresponsive polymer layers:
   - **a)** self-supporting layers **[H1, H2, H12]**
   - **b)** layers based on polyglycidol **[H5],** poly[oligo(ethylene glycol) methacrylate] **[H4, H6]** and (co)poly(2-substituted-2-oxazoline)s **[H7, H8, H9]** immobilized on supports
4. An estimation of the influence of polymers composition and structure and properties of thermoresponsive polymer layers on the ability of cell sheet culture and detachment taking into account:
   - **a)** an adhesion, proliferation and detachment of cell sheet **[H1, H4, H5, H7, H8]**
   - **b)** transfer of cell sheet **[H8, H11]**
   - **c)** cell characterization after culture on thermoresponsive polymer layers **[H7]**

All of the works described in this dissertation are focused on the ability to control the synthesis of polymers and their grafting with the support in order to influence the properties of the resulting polymer layers. Particular emphasis was placed on determining the effect of the polymer layers properties on their capability to reduce protein adsorption or to culture of cell sheets. Such analysis enabled, within the presented habilitation work, to define the potential use of received materials in reconstructive medicine and tissue engineering.

A monothematic articles [H1-H12] described in this dissertation are collective works in which the role of each author is defined and described in the table in Chapter 4B of this summary.

2. HYDROPHILIC POLYETHER LAYERS FOR REDUCING PROTEIN ADSORPION

The uncontrolled adhesion of biological compounds on the surface of biomaterials is a harmful phenomenon. Medical implants, regardless of their structure, will become coated with a layer of proteins within a few seconds of contact with physiological fluids and tissues. As a result, the host defense mechanisms is activated, which can lead to inflammatory reactions, thromboembolic complications or deterioration of the functioning of the device [13]. Therefore, materials with antifouling properties have been the subject of much interest and extensive research within the last few years [14-16]. This antifouling behavior is usually achieved by coating the surface with polymer layer with appropriate properties. Generally, two major classes of polymers have been investigated for the minimization of nonspecific adsorption: hydrophilic and zwitterionic. Among these polymers, the most widely studied layers are based on poly(ethylene glycol) [3] which is non-toxic, non-immunogenic and water-soluble. Polyglycidol (PGl) – a poly(ethylene glycol) analogue, is also a biocompatible, hydrophilic polymer and additionally possess functional groups capable for further modification. It can therefore be a polymer suitable for this kind of application.

In the author labs, methods for controlled polymerization of glycidol and its derivatives to obtain polymers with linear [17], branched [6], dendritic of "pom-pom" type [18] or "bottle-brush" [19] structure have been developed. Despite the high level achieved in polyglycidol synthesis and in attempts to apply it in biomedicine [20], few data on its use as an antifouling coating have been published at the time of the habilitation work [7-9]. These studies concerned only dendritic polyglycidol, so it was not possible to determine the relationship between the polymer structure, the polymer layer structure and properties, and the resistance of obtained materials for protein adsorption.

In the habilitation work, the research issues related to the development of polymer layers based on linear polyglycidol and its copolymers with ethylene glycol capable for reducing protein adsorption have been taken [H3, H10].

2.1. Immobilization of linear polyglycidol – synthesis and characterization of polymer layer [H10]

Polymer surfaces containing a layer of polyglycidol or its block copolymers with ethylene glycol of linear structure (scheme in Fig. 1.2), described in [H10], were obtained using a grafting-to method. The synthesis involved two steps: modification of solid supports (silica) to introduce reactive functional groups followed by immobilization of previously synthesized (co)polymers of glycidol.

The scheme of silica support modification is shown in figure 2.1.

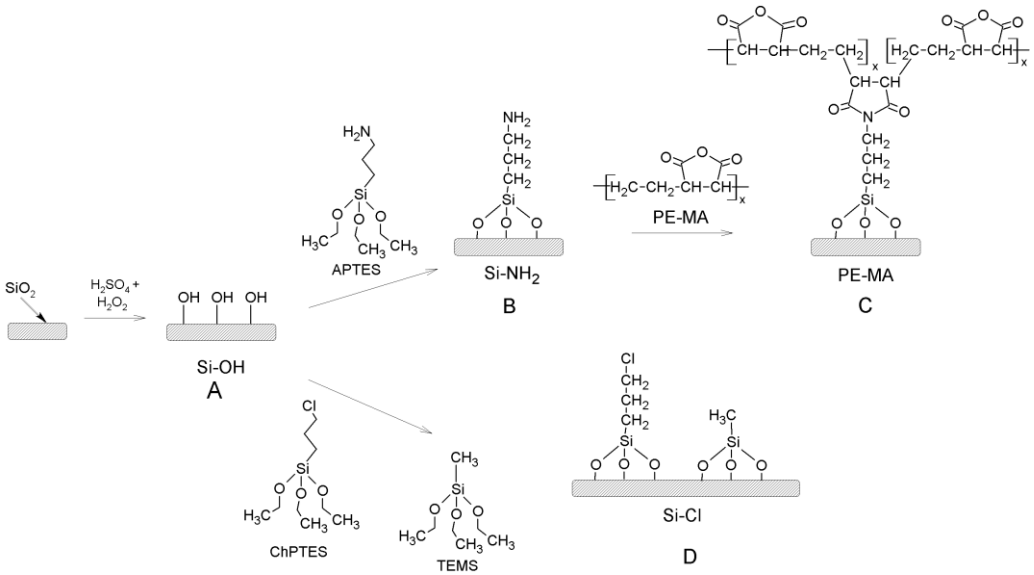


Fig. 2.1. Modification of silica supports: (A) hydroxylation; (B) and (D) silylation; (C) bonding of PE-MA intermediate layer [H10].

Modification of silica supports was performed through hydroxylation (Fig. 2.1 A), followed by reaction with organosilicon compound ((3-aminopropyl)triethoxysilane – APTES or (3-chloropropyl)-triethoxysilane – ChPTES; Fig. 2.1 respectively B and D). The amount of reactive chloropropyl groups on the support (Si-Cl), and thus the graft density, was controlled by the molar ratio of the active ChPTES compound to the inactive organosilicon compound - triethoxymethylsilane (TEMS) (ranging from 1 to 0.1 mol). Supports containing amino-groups (Si-NH_2) were further modified with copolymer of ethylene and maleic anhydride (PE-MA) to obtain so-called intermediate layer (Fig. 2.1 C).

The presence of the reactive functional groups on the surfaces allowed for immobilization of the (co)polyglycidols via the grafting-to technique. By using the procedures previously developed at the habilitant lab [21], linear polyglycidols of low (LPG_L, LPG_{LT}) and high (LPG_H) molar mass were prepared by anionic and coordination polymerization of glycidol with protected hydroxyl group (ethoxyethyl glycidyl ether). Copolymers of glycidol and ethylene glycol ($L(EO\text{-}PG)_{LT}$) were prepared by anionic polymerization of glycidol with protected hydroxyl group initiated by the poly(ethylene glycol) macroinitiator (M_n = 350 g/mol (PEG_{350})). The molar masses and dispersities of the synthesized polymers were determined by GPC system containing a multi-angle light scattering detector. The results are summarized in Table 2.1.

Table 2.1. Characteristic of linear (co)polyglycidols [H10]

Polymer designation in publication	Polymer	M_n [g/mol]	M_w/M_n
LPG_H	polyglycidol	$1.9 \cdot 10^6$	1.40
LPG_L		$8 \cdot 10^3$	1.04
LPG_{LT}		$8 \cdot 10^3$	1.04
$L(EO\text{-}PG)_{LT}$	copolymer of glycidol and ethylene glycol	$6 \cdot 10^3$	1.10

The obtained and characterized polymers of glycidol were then immobilized on modified silica supports (Fig. 2.2).

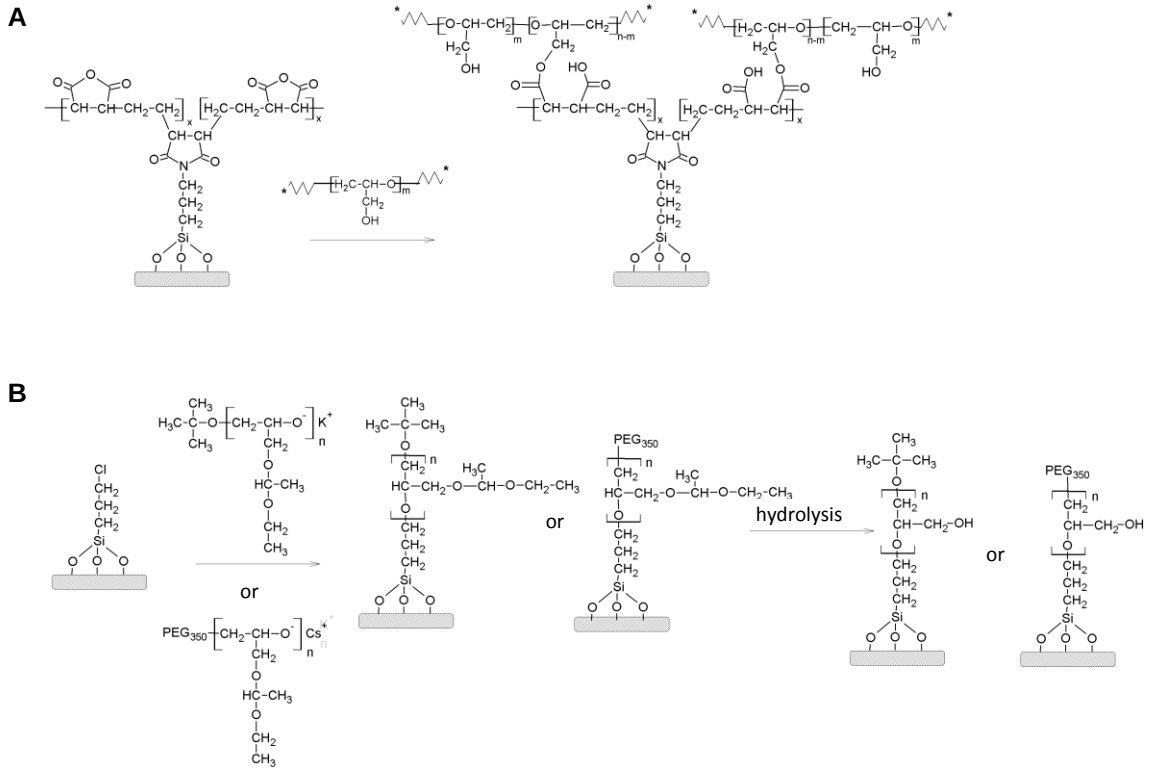


Fig. 2.2. Scheme showing the covalent bonding of polymers of glycidol with modified supports through (A) the reaction of functional groups present on the polymer chain or (B) the termination of the living polymer chain [H10].

Two types of polymer layers were obtained: with the structure of interpenetrating polymer chains that are multiply attached to the surface (Fig. 1.2 I and 2.2 A) and with the polymer brush structure (Figures 1.2 II and III and 2.2 B).

In the first case polyglycidols of high (LPG_H) and low (LPG_L) molar mass polyglycidols were covalently bound with the silica support via reaction between the hydroxyl groups of the polymer (distributed along the polymer chain) with the functional anhydride groups of the substrate derived from PE-MA (Fig. 2.2 A). For this purpose, polyglycidol solutions in methanol with different concentration (0.5 to 10 %) were spin-coated on a modified silica support in order to obtain polymer layers of different thicknesses. The resultant polymer surfaces were marked as follows: $LPG_{H\_0.5\%}$, $LPG_{H\_1\%}$, $LPG_{H\_2\%}$, $LPG_{H\_3\%}$ and $LPG_{L\_3\%}$, $LPG_{L\_10\%}$.

In the second case grafting of the polymer was achieved through termination of the living polyglycidol chains with protected hydroxyl groups or living copolymer of glycidol (with protected hydroxyl groups) and ethylene glycol chains by the chloropropyl terminating groups anchored on the support (LPG_{LT} and $L(EO\text{-}PG)_{LT}$, respectively; Fig. 2.2 B). By using silica supports with a variable number of reactive Si-Cl groups, the grafting density of polymer chains was altered. The obtained polymer surfaces were designated as LPG_{LT_1}, $LPG_{LT\_0.5}$, $LPG_{LT\_0.1}$, $L(EO\text{-}G)_{LT\_1}$ and $L(EO\text{-}G)_{LT\_0.1}$.

The presence of the (co)polyglycidol layer covalently bound to the surface was confirmed by FT-IR analysis (for structures with interpenetrating polymer chains repeatedly attached to the surface – Fig. 2.3) and by X-ray photoelectron spectroscopy (for polymer brushes – Fig. 2.4).

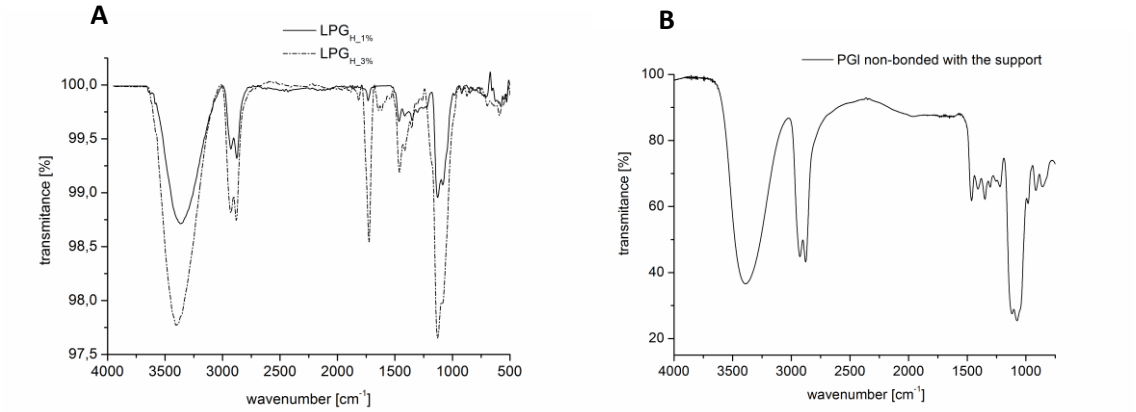
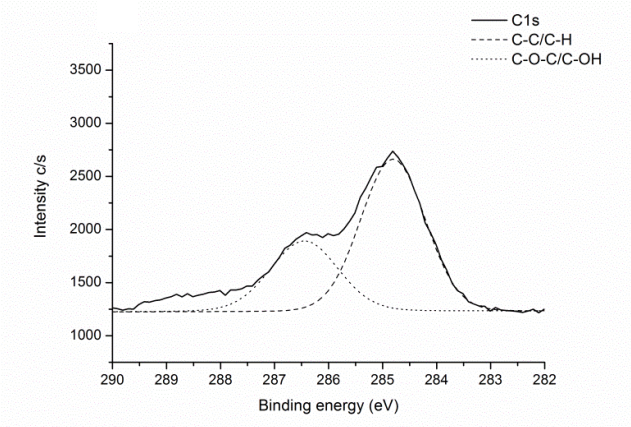


Fig. 2.3. FT-IR spectra of (A) exemplary polyglycidol layer immobilized on the substrate and (B) polyglycidol not attached to the substrate for comparison [H10].



Surface designation in publication	C1s	O1s	Si2p	Cl2p	C/O
Si-OH	19.0	37.0	44.0	0.00	0.51
Si-Cl	22.3	37.0	39.7	1.00	0.60
$L(EO\text{-}G)_{LT\_1}$	53.4	23.1	23.5	0.00	2.31

Fig. 2.4. XPS analysis of exemplary polymer layer with polymer brush structure: high resolution C1s spectra for $L(EO\text{-}G)_{LT\_1}$ layer and in table the surface composition for support modified with ChPTES and with $L(EO\text{-}G)_{LT\_1}$ polymer [H10].

On FT-IR spectra made for polyglycidol layer, the characteristic absorption bands derived from groups present in this polymer are visible (Fig. 2.3). An additional adsorption band at 1730 cm^{-1}, corresponding to the stretching vibrations of C=O groups from the ester groups, indicates that the hydroxyl groups from polyglycidol were reacted with anhydride groups from the intermediate PE-MA layer, thus confirming the chemical bonding of the polyglycidol to support.

In the case of layers with polymer brush structure, quantitative analysis of the surface composition performed by the XPS technique revealed anchoring on the silica supports both the chloropropyl groups as well as the polyglycidol chains. This is revealed by the presence of a chlorine signal after the modification reaction and its disappearance after the termination reaction with the live polyglycidol chains. After the termination reaction, a characteristic C-O-C peak at 286.4 eV (Fig. 2.4), an increase in the C/O ratio and a decrease in silica concentration are also observed.

The grafting-to techniques presented in this work do not require any modification of the polyglycidol chains, what distinguish such procedures from the methods previously reported [22, 23]. Additionally, they provide a fast and easy way for covalent immobilization of well-defined linear polyglycidol of different molar masses with a solid substrate. In the case of polyglycidol layer with polymer brush structure, a novel and an alternative way was applied to synthesis them – termination of living chains.

2.2. The properties of polyglycidol layers and their ability to reduce the protein adsorption [H10]

An essential issue, within the frame of surfaces reducing protein adsorption, was the estimation of relationship between the polymer layers structure and properties and their possibility to reduce the protein adhesion.

Therefore, the layer morphology, its roughness (RMS - average roughness coefficient), thickness (h) and affinity for water were correlated with the amount of protein adsorbed on the polymer surface.

The layer parameters achieved during atomic force microscopy (AFM), ellipsometry and contact angle measurements (θ) were obtained for layers in the dry state and after incubation in water and are presented in Table 2.3.

Table 2.3. Characteristics of linear (co)polyglycidol layers [H10]

	surface designation in publication	Contact angle (°)			Layer thickness h (nm)	Grafting density σ (chain/nm^2)	RMS [nm]
		Θ_1 (dry layer)	Θ_2 (water 20 °C)	Δ_Θ (Θ_1-Θ_2)			
Layer with a structure of interpenetrating polymer chains, multiply attached with the surface	$LPG_{H\_0.5\%}$	78±2	73±2	5	15±0,3	[a]	0.15
	$LPG_{H\_1\%}$	72±1	65±1	7	31±0,3		0.25
	$LPG_{H\_2\%}$	64±2	55±2	9	61±0,5		0.39
	$LPG_{H\_3\%}$	55±1	43±2	12	120±0,7		0.41
	$LPG_{L\_3\%}$	54±1	52±1	2	7±0,3		0.42
	$LPG_{L\_10\%}$	50±1	43±1	7	140±0,7		0.21
Layer with a structure of polymer brush	LPG_{LT_1}	34±1	33±1	1	1.5±0.3	0.113	0.30
	$LPG_{LT\_0.5}$	30±1	28±1	2	1.4±0.3	0.105	0.15
	$LPG_{LT\_0.1}$	32±1	31±1	1	1.1±0.3	0.083	0.10
	$L(EO\text{-}G)_{LT\_1}$	33±1	33±1	0	2.3±0.3	0.230	0.15
	$L(EO\text{-}G)_{LT\_0.1}$	31±1	30±1	1	1.7±0.3	0.170	0.15

[a] not determined due to the specific bounding of polymer with support

An AFM analysis showed, that all of the surfaces obtained are characterized by a smooth surface (RMS from 0.1 to 0.42 nm, Table 2.3). A slightly increase of RMS with the increase of polymer layer thickness and grafting density was observed leading to a more irregular surface texture.

The thickness of linear polyglycidol layers ranged from 1.1 nm to 140 nm [H10]. It was depended on the concentration of polyglycidol solution used during polymer immobilization, its molar mass, the method of binding the polymer with the support and on the grafting density. For polyglycidol layers with a structure having interpenetrating polymer chains, multiply grafted to the support (LPG_H or LPG_L), the layer thickness increased with increasing polymer concentration and molar mass. Polymer layers with a structure of polymer brush were characterized by a small layer thickness that increased from 1.1 to 1.5 nm for the LPG_{LT} series and from 1.7 to 2.3 nm for the $L(EO\text{-}G)_{LT}$ series with an increase of a number of terminating groups on the support. The polymer grafting density for this type of surfaces was 0.105-0.230 chains/nm^2.

The affinity for water of the obtained polymer layers was determined by measuring the contact angles for dry and incubated in water at 20 °C surfaces. The obtained angles ranged from 30 ° to 78 °, depending on the composition of the immobilized polymer and structure of the polymer layer. The lowest value of θ was obtained for surfaces coated with polyglycidol of polymer brush structure.

The polyglycidol layers with a properties that are described in Table 2.3 were used for protein adsorption studies. These studies were conducted in cooperation with the Department Molecular Biology and Genetics of Medical University of Silesia in Katowice (SUM). As a model protein, a human plasma fibrinogen (conjugated to fluorescent dye) was used. This protein plays a key role in blood coagulation. The fluorescence intensity of the protein adsorbed on the obtained surfaces was measured. The results are shown in figure 2.5.

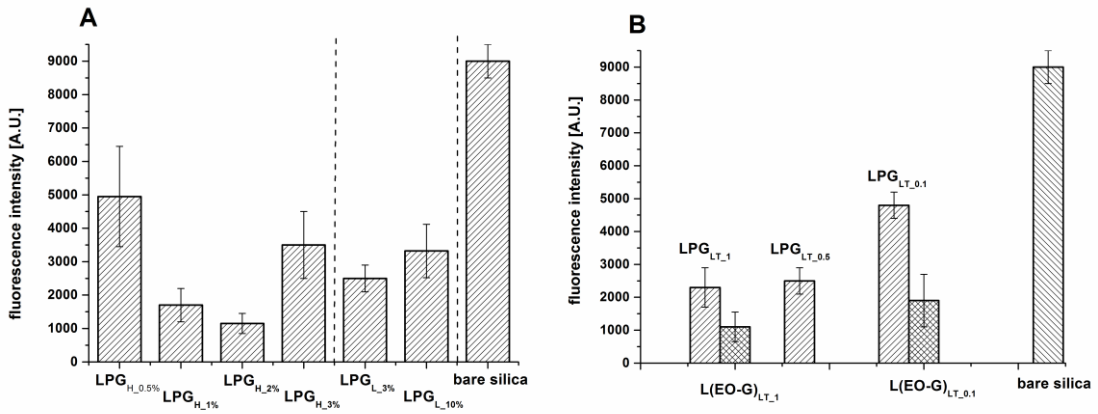


Fig. 2.5. Fluorescence intensity graph of fibrinogen adsorbed on the surfaces covered with (A) linear polyglycidol with a structure of interpenetrating chains multiply grafted with the support and (B) linear polyglycidol with a structure of polymer brush [H10].

The results indicate that although fibrinogen quickly formed an adsorbed layer on the untreated/bare silica substrates, its unspecific adsorption was effectively suppressed by functionalization of the surfaces with linear polyglycidol. It was observed, that the fluorescence intensities of the fibrinogen adsorbed on the surfaces modified with polyglycidol were approximately 45-90 % (assuming all series) lower than those on the bare silica surface.

The amount of protein adsorbed on polyglycidol layers (Fig. 2.5 A) and on layers of its copolymer with ethylene glycol (Fig. 2.5 B) depended on the molar mass of the immobilized polymer, film thickness, grafting density and method of polymer binding with the substrate [H10]. LPG_L surfaces with lower molar mass and structure shown in Figure 1.2 I show lower ability to resist fibrinogen deposition than those coated with high molar mass polyglycidol (LPG_H). For LPG_H series, it was shown that with the increase of the polymer layer thickness, the fibrinogen adsorption decreases. However, the adsorbed fibrinogen reached a minimum value at a certain polymer layer thickness, above which it increased again ($LPG_{H\_3\%}$). Such dependence was also observed for the surfaces covered with low molar mass polymer ($LPG_{L\_10\%}$). In the case of both polymer layers, polymer chains formed probably a multilayer coating that is loosely bound with the silica support leading to its irregularity. This caused the protein to penetrate within the layer and adhere on a support. Similar behavior was observed in other works [24, 25].

For surfaces with the polymer brush structure (LPG_{LT} and $L(EO\text{-}G)_{LT}$), the fibrinogen adsorption increased with a decreasing grafting density. This dependence can be explained by using a model describing the degree of chain overlap on the surface (degree of surface coverage) [26]. It takes into account the distance between the grafted chains and the radius of gyration of polymer. Only for the $LPG_{LT\_0.1}$ surface (with the lowest grafting density), the degree of surface coverage of polymer chains is insufficiently high, what cause the presence of open spaces between the chains. This led to the penetration of the protein into the polymer layer and its adsorption on the support. When poly(ethylene glycol) was introduced into the polyglycidol chain, the resultant surfaces ($L(EO\text{-}G)_{LT}$) exhibited better antifouling properties compared with the surfaces covered only with polyglycidol (LPG_{LT}). By comparing the adsorption of fibrinogen onto all surfaces based on linear polyglycidol obtained in this work, it has been observed that surfaces with a structure of polymer brush the most effectively reduce the amount of adsorbing protein. This is due to the tightly packed arrangement of polymer chains.

2.3. Conclusions

- The use of grafting-to method led to the formation of polyether layers, based on linear homopolymers or its copolymers with ethylene glycol, of different structure (polymer chains multiply attached to the support or polymer brush) with different molar masses and different grafting densities
- An affinity for water and thickness of polyglycidol layers were depended on the molar mass of the immobilized polymer, composition, method of its binding with the support and on grafting density
- A relationships between the composition of immobilized polymers and their binding with the support, polymer surface properties (affinity for water, layer thickness, morphology) and their resistance to protein adsorption were determined
- Well-defined linear (co)polyglycidol layers on silica supports have been obtained. It was established that fibrinogen adsorption on surfaces was reduced by approximately 45-90 % compared with the bare silica supports. From all investigated surfaces, those covered with high molar mass polyglycidol as well as the surface covered with a (co)polymer of ethylene glycol and polyglycidol at the highest grafting density turned out to be the most effective for preventing absorption of fibrinogen

3. THERMORESPONSIVE POLYMER LAYERS FOR TISSUE ENGINERRING

The studies described so far have shown that it is possible to obtain hydrophilic polymer layers based on polyglycidol that efficiently reduce protein adsorption. **That is why, it was interesting to determine the ability to control the affinity for water of the obtained polymer layers (so called hydrophilic-hydrophobic balance) and also the ability to "switch" this affinity depending on the external conditions.** Such behavior of coatings can be used for controlled cell adhesion. It is known that the cells more likely adhere to hydrophobic surfaces, while hydrophilic layers resist their adhesion. Thus the obtainment of layers with switchable affinity for water opens the way for their use in tissue engineering.

Substrates made of modified polystyrene (TCPS) are generally used for cell cultures. Good cell adhesion to these substrates is achieved by appropriate control of their hydrophilic-hydrophobic balance. Separation of proliferated cells from such surfaces is traditionally done enzymatically what however, destroy a number of cells and disturb the cell sheet integrity. In that case, a single cell suspension is obtained. The use of such a suspension in regenerative medicine is common. However, some restrictions have been observed in its application, for example, difficulties in controlling the location of introduced cells or the formation of cells aggregates often not attached to the host tissue. In the case of scaffolds, that are populated by cell suspension, an inflammatory responses are often triggered or in the case of biodegradable scaffolds the products of their degradation are toxic. In many cases, these problems could be avoided by culturing the cells in the form of a sheets followed by separating them from the scaffold and transferring to the desired place in an intact form.

Polymers sensitive to temperature changes (so-called thermoresponsive polymers) can be used to develop such scaffolds. A temperature change allows to change the affinity for water of such polymers. They are soluble below a certain temperature and, when it is exceeded (so-called phase separation temperature – T_{CP}), polymer precipitate as a result of strong intra- and intermolecular interactions. The immobilization of thermoresponsive polymers on the support lead to surfaces with properties, such as affinity for water, morphology and thickness, that can be reversible changed by the temperature alteration [27].

At the beginning of habilitation work, thermoresponsive substrates based mainly on poly(*N*-isopropylacrylamide) (PNIPAM) and its copolymers were used for cell sheets culture and their non-invasive detachment. This idea, called cell sheet engineering, was developed by T. Okano [11]. The cell culture is carried out at a temperature above the phase separation temperature of the thermoresponsive polymer, when the surface is hydrophobic. When the cells form a sheet, the temperature is lowered, the surface becomes hydrophilic and the cell sheet spontaneously separates from the substrate. It is not necessary to use enzymatic methods of cell separation.

Initially, the attention has been focused on PNIPAM-based polymers. These polymers are characterized by a T_{CP} around the physiological temperature which makes them attractive for biomedical applications. However, PNIPAM does not have functional groups available for modifications, its phase transition curves show a hysteresis, it can aggregate and additionally it irreversibly interacts with biological compounds, e.g. amino acids or proteins. While a lot of studies on intelligent polymers have been made thus many thermoresponsive polymers have been identified, in many respects more promising [28]. In the laboratory of Nano- and Microstructural Materials CMPW PAN, a modification of polyglycidol to thermoresponsive copolymers with T_{CP} ranging from 10 °C to 90 °C

was developed (with my leading participation) [10, 29]. By appropriate modification, it is possible to control the affinity for water of obtained the glycidol copolymers only by changing the environmental temperature.

By using the obtained knowledge, **the studies on the development of polymer layers based on modified polyglycidol, with affinity for water controlled by a temperature, were undertaken in the habilitation work. The determination of interaction with cells of such obtained polymer layers was of significant importance.** The researches have also been extended to other thermoresponsive polymers: poly[oligo(ethylene glycol) methacrylates] and poly(2-substituted-2-oxazoline)s. The aim of the study was to determine the suitability of these layers for culture and non-invasive detachment of the cells in a form of a sheet.

By using grafting-from and -to techniques, a thermoresponsive polymer layers immobilized on a solid (glass or silica) supports were obtained. Works to determine whether thermoresponsive layers of sufficient mechanical parameters in a form of self-supporting layers (without immobilizing the polymer on the support) have also been undertaken.

For the clarity of the dissertation, syntheses and properties of thermoresponsive polymer layers, both self-supporting and immobilized on the substrate will be described first (chapters 3.1 and 3.2). Section 3.3 will be related to the determination of the influence of the polymer composition and structure and the properties of the thermoresponsive surface on the ability of the cell adhesion and detachment.

3.1. Thermoresponsive self-supporting layers based on polyglycidol – synthesis and properties [H1, H2, H12]

Thermoresponsive self-supporting layers (in form of polymer gels) based on polymers of glycidol were obtained using photocrosslinking. As compared to chemical crosslinking, this method of gels preparation is simple and does not require the use of many reagents. The aim of the work was to develop the conditions of photocrosslinking of polyglycidol in such a way that thermoresponsive layers with optimum mechanical parameters will be obtained.

The work with the use of UV-radiation required first an estimation of the UV effect on the stability and possible degradation of the polyglycidol chain. This issue is also important because this type of radiation is used in sterilization processes of biomaterials. The behavior of polyglycidol under UV irradiation and its possible photodegradation have not been studied so far, so in [H2] this issue has been undertaken.

At work [H2], the behavior of aqueous polyglycidol solutions (at different concentrations) was determined by exposure to UV radiation. A changes in physical and chemical properties of polymer was monitored by gel permeation chromatography (SEC-MALLS), FT-IR and NMR spectroscopy.

It was observed that during irradiation, the shape of the chromatograms changed from monomodal to bimodal and the quantity of the lower molar mass fractions increased with an increase in the photodegradation time. The molar masses for the both maxima of bimodal chromatograms differed in half. In all of the chromatograms, the lack of signals at higher elution volumes pointed to the absence of low molar mass oligomers as photodegradation products (Fig. 3.1 A). This could indicate that UV irradiation of polyglycidol solutions led to the fragmentation of the polymer

chains, which occurred through scission around the midpoint of the polymer chain but not at the polymer chain end.

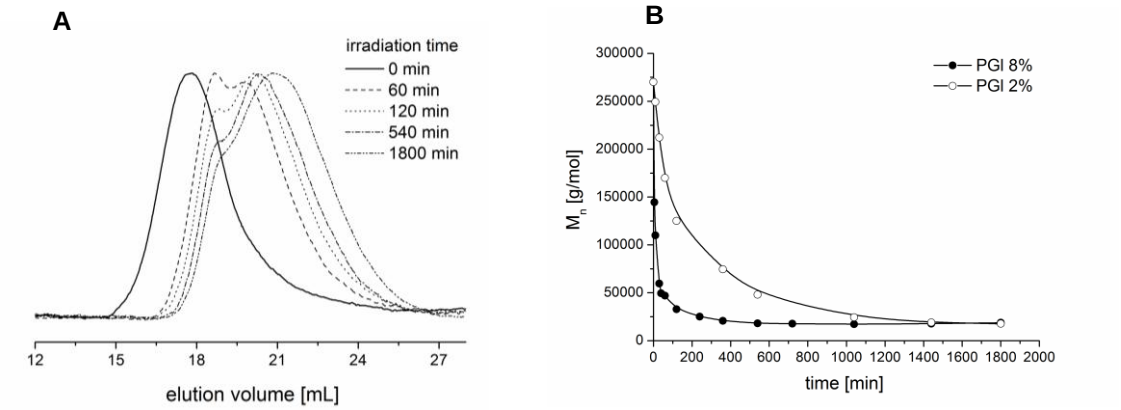


Fig. 3.1. (A) Chromatograms of polyglycidol and the products formed after its UV irradiation; (B) changes in the molar mass of polyglycidol as a function of the irradiation time (for different polymer concentration).

The molar masses of polyglycidol decreased significantly as a function of irradiation time. For higher polymer concentration, the molar mass decreased to half of its initial value after 10 minutes of UV radiation. Regardless of the polymer concentration, the molar mass of the degraded polyglycidol approached a limiting value of 17 000 g/mol.

During the irradiation of the polymer solutions, a strong acidification was observed. The pH of the solutions decreased from 7 to 3. This decrease indicates that the number of protons (H^+) from acid groups originated via degradation were released into the solution. FT-IR, ^{1}H NMR and ^{13}C NMR analysis showed that after irradiation the resulting degradation products contained ester, α-hydroxyester and formate groups.

Based on the obtained data a mechanism of the photodegradation of polyglycidol was proposed [H2] (Fig. 3.2).

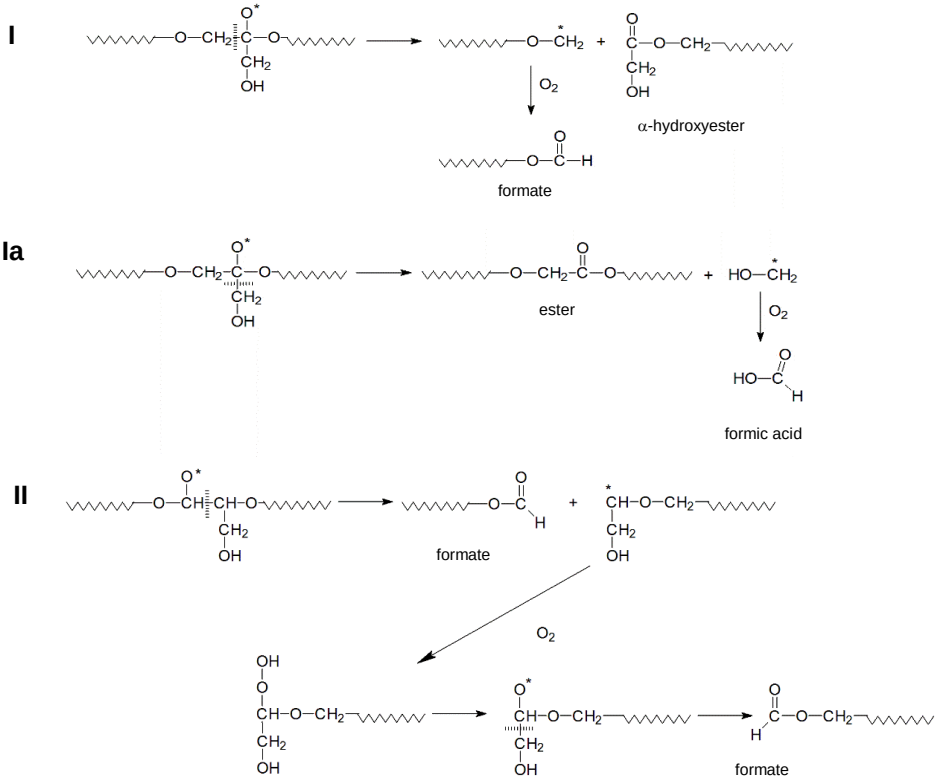


Fig. 3.2. Proposed mechanism of the polyglycidol degradation under UV irradiation [H2]. Polymer chain scission during the decomposition of the radicals formed on tertiary (I and Ia) and secondary (II) carbon atom.

The photodegradation of polyglycidol begins with the formation of hydroxyperoxides at the carbon atom of the methylene groups in the α-position relative to the oxygen atom of the ether group. Hydroxyperoxides decompose and the macroalkoxy radicals are formed. The formation of alkoxy radicals on both tertiary and secondary carbon atoms was taken under consideration (Fig. 3.2 I i Ia). The macroalkoxy radicals undergo decomposition via β-scission reactions between carbon atoms in the polymer main chain and in the side groups. This lead to the formation of ester groups in the polymer chain and to the α-hydroxyester and formate groups at the polymer end-chain (Fig. 3.2). Formates, esters and α-hydroxyesters can hydrolyze in water which leads to the formation of formic acid ions and hydroxyl end groups on the polymer chain (thus the solution becomes acidic).

In work [H2] it has been shown, that the exposure of polyglycidol to UV irradiation at 254 nm results in the degradation of this polymer. It has been established, that UV generates radicals on the polyglycidol chain that under certain conditions cause the polymer chain scission. The radicals generated on the polymer chain can also recombine under appropriate conditions, leading to polymer chain crosslinking and gel formation [30]. The above assumption was used in work [H1, H12] where, in the presence of photosensitizer activated by the UV radiation, a crosslinking of polyglycidol derivatives was performed.

Polyglycidol and polyglycidol whose hydroxyl groups were modified with ethyl isocyanate [10] were used for crosslinking. Poly(glycidol-co-ethyl glycidyl carbamate)s (PGl_URE) with varying degrees of substitution of hydroxyl groups with hydrophobic groups and therefore with different T_{CP} were used (Table 3.1). For photocrosslinking benzophenone (BP) and its derivative (4-benzoylbenzyl) trimethylammonium chloride (BBTMAC) at different concentration (2 %, 5 % or 10 %) were applied

as photosensitizers. The polymer/photosensitizers films were prepared by film-casting and then subjected to UV irradiation [H12].

Table. 3.1. Characteristics of polymers used for photocrosslinking [H1, H12]

polymer designation in publication	$M_{n,SEC}$ [g/mol]	M_w/M_n	[OH]:[EtIz]	Degree of modification [%]	T_{CP} [^oC]
PGl	$1.25 \cdot 10^6$	1.37	-	-	[a]
PGl_URE1	$1.45 \cdot 10^6$	1.40	1:0.41	40	28
PGl_URE2	$1.40 \cdot 10^6$	1.40	1:0.38	37	33
PGl_URE3	$1.30 \cdot 10^6$	1.57	1:0.34	32	46

[a] polymer completely soluble in water in a measurable range

Upon absorption of a photon with a wavelength of 254 nm, BP or BBTMAC undergo a photo-dissociation into a radicals that are able to generate the radicals on a polymer chain. The recombination of radicals and subsequent production of carbon-carbon bonds between the polymer chains cause the crosslinking of these polymers (Fig. 3.3).

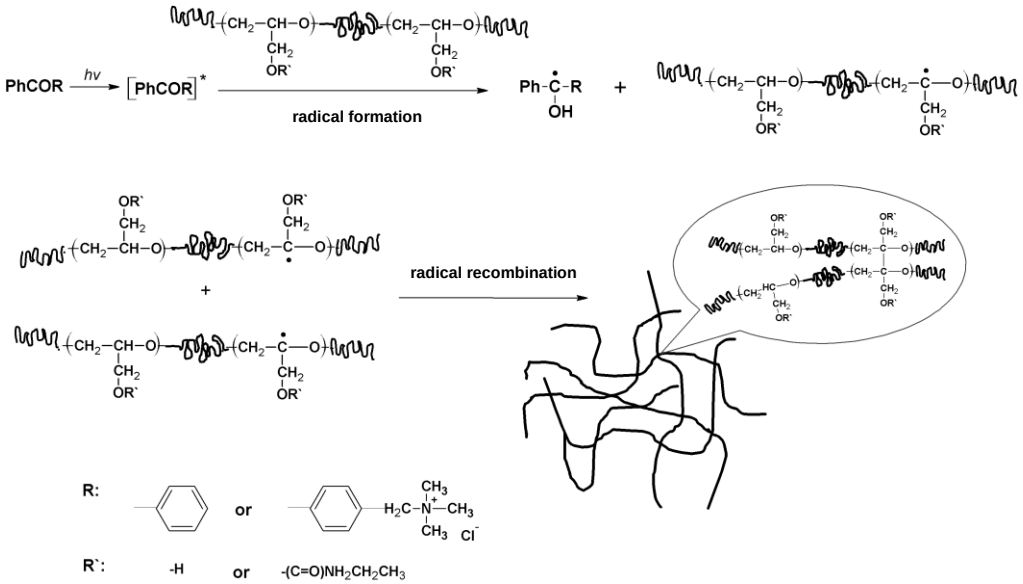


Fig. 3.3. Simplified scheme of the photocrosslinking of polyglycidol and its derivatives [H12].

The influence of the composition of the starting copolymer and the type and concentration of photosensitizer on the crosslinking efficiency (referred as the amount of gel fraction) and the degree of gel swelling at room and elevated temperatures was determined.

The amount of gel fraction, regardless of the type of photosensitizer used, decreased with the photosensitizer concentration. It was observed, that the best crosslinking efficiency (90 % for both photosensitizers) was achieved at low photosensitizer concentration applied. The crosslinking efficiency for PGl_UREs is significantly lower than that for pure polyglycidol. The amount of gel fraction is then only about 35 %, regardless of the type of photosensitizer. The presence of the hydrophobic ethyl

carbamate groups in the polymer chain seems to prevent the formation of radicals on the polymer backbone, thus suppressing photocrosslinking.

A relatively low crosslinking efficiency for PGl_URE and an atypical behavior associated with a decrease in gel fraction with an increase in the amount of photosensitizer was observed. The studies of the soluble gel fraction indicated that the molar mass of the polymers was reduced and the molar mass distribution increased. The presented results suggest that the UV irradiation of PGl and PGl_URE films led to their crosslinking; however, this process competes with the process of degradation of the polyether chains [H12].

In [H12] an optimal crosslinking parameters have been also determined for which the degradation is minimal and the obtained gels are characterized by a relatively high degree of crosslinking. The behavior of these materials in water at temperatures ranging from 25 °C to 75 °C (example data in Fig. 3.4) has been investigated, which is important because of their potential use as a cell culture surfaces.

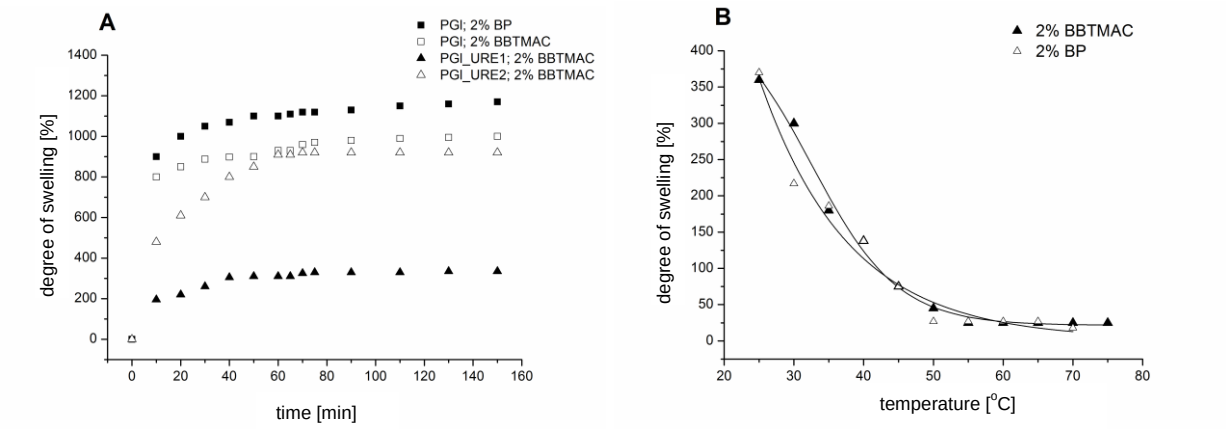


Fig. 3.4. (A) Swelling kinetics of exemplary gels obtained during photocrosslinking of polyglycidol and its derivatives; (B) Swelling degree of gels obtained during photocrosslinking of PGl_URE1 as a function of temperature.

The equilibrium swelling degrees of the PGl hydrogels are in a range from 900 to 1700 % and depended on the amount of the photosensitizer. It can be observed that gels crosslinked with BBTMAC showed slightly lower swelling degrees than those crosslinked with BP. Gels derived from modified polyglycidol (PGl_URE series) swell lower than gels based on polyglycidol (Fig. 3.4 A). This is due to the presence of a lower number of highly hydrophilic hydroxyl groups, which limits the formation of hydrogen bonds between the polymer chains and water molecules, resulting thus in lower water absorption.

The gels obtained during photocrosslinking of PGl_URE were thermoresponsive. They showed a volume phase transition (a decrease of swelling degree with temperature), although within a broad temperature range (exemplary data in Fig. 3.4 B). The volume phase transition (T_{VPT}) for PGl_URE1 was 30 °C (irrespective of the type of photosensitizer), for PGl_URE2 35 °C (irrespective of photosensitizer type) and 40 °C (BP) or 45 °C (BBTMAC) for PGl_URE3.

It has been observed that some gels were not enough mechanically stable. During swelling or shrinking they fragmented into smaller pieces. In addition, because the gel formation was accompanied by the polymer degradation and the gel response to temperature change was slow, it was decided not to use the received gels for studies of their interaction with cells.

In order to increase the mechanical strength of thermoresponsive gels, to improve their behavior in water under temperature changes and above all to eliminate the degradation of the polymeric chain under UV irradiation, in habilitation work [H1] the photocrosslinking process was carried out for a water polymer/photosensitizer mixture after its freezing. So called cryogels were then formed. During freezing, water forms ice crystals, whereas soluble substances accumulate in a non-frozen liquid microphase. The irradiation with UV lead to the crosslinking and gel formation in this microphase and the ice crystals act as porogens. Therefore, the exposure time can be minimized thus limiting the possible degradation of the polymer. Additional cryogels, compared to conventional gels, exhibit a much faster reaction to hydration and dehydration.

Polyglycidol and its thermoresponsive derivatives PGl_URE1, PGl_URE2 and PGl_URE3 described earlier (Table 3.1) were frozen in the presence of a BBTMAC photosensitizer and then shortly exposed to UV radiation. The mechanism of polymer crosslinking in the non-frozen liquid phase is the same as in the case of photocrosslinking without freezing (Fig. 3.3). In the [H1] work, the effect of copolymer composition, concentration during crosslinking and exposure time on the efficiency of cryogel formation (gel fraction) and properties (equilibrium swelling degree and T_{VPT}) was investigated.

Cryogels with a yield of 73-88 % were obtained. They were characterized by a relatively high swelling degree in water. The cryogels obtained from polyglycidol swelled up to 7000 % while the thermoresponsive cryogels PGl_URE up to 5200 %. The lower degree of swelling of PGl_URE materials was attributed to the presence of hydrophobic ethyl carbamates groups in the polymer chain.

The PGl_URE cryogels were thermoresponsive (Fig. 3.5). Their degree of swelling decreased with the temperature increase. The composition of the starting copolymer, subjected to photocrosslinking, significantly influenced the value of the T_{VPT}. The higher the content of hydrophobic groups in the copolymer, the lower the value of the T_{VPT} (Fig. 3.5 A). The cryogels responded very quickly to temperature changes (Fig. 3.5 B). Such ultra-rapid swelling and shrinkage changes were associated with their macroporous structure.

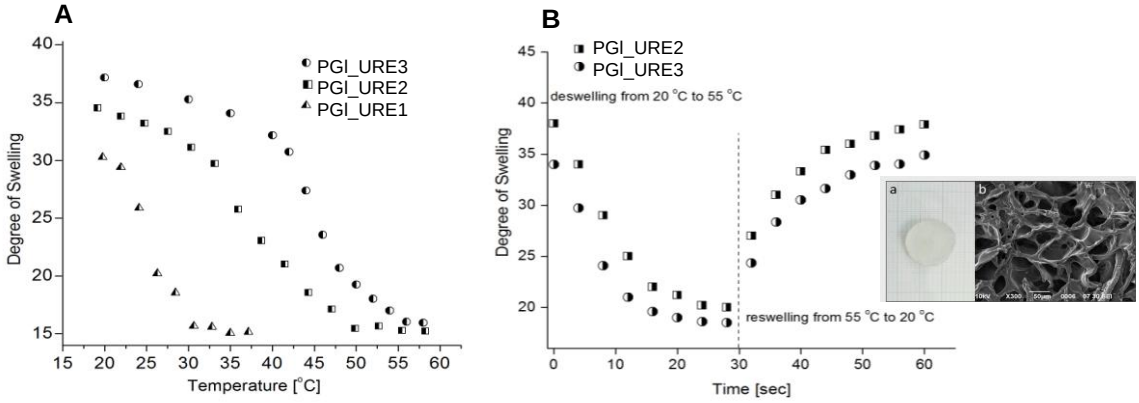


Fig. 3.5. (A) The changes of the swelling degrees for cryogels obtained from thermoresponsive PGl_URE of different composition as a function of temperature; (B) Deswelling–reswelling kinetics of PGl-URE cryogels.

PGl_URE cryogels exhibited cyclic swelling-deswelling changes as a function of temperature, which is an advantageous due to their reusability. It was found that cryogels reached the same degree of swelling until fifth cycle. This means that the material is able to reconstruct its internal structure and volume after changes from swollen to shrunk state without loss of its mechanical properties.

On the base of performed studies, an optimum exposure time of UV radiation and concentration of polymer, required to obtain cryogels with high amount of gel fraction, high swelling rate and rapid reaction to temperature changes, were determined. These cryogels were therefore used as a self-supporting layers for cell culture.

The research described in the work [H1, H2, H12] allowed for:

- determination of UV radiation limit parameters (such as concentration of polymer, UV exposure time) under which the polymer chain scission occurs. The obtained results should be taken into account when planning the potential application of polyglycidol
- obtaining a self-supporting layers of polyglycidol and its thermoresponsive derivatives (poly(glycidol-co-ethyl glycidyl carbamate)s) during the UV irradiation of a photosensitizer/polymer mixture. The obtained gels were thermoresponsive and their T_{VPT} values can be controlled by the composition of the polymer. These gels, however, were not mechanically stable, besides during their irradiation the polymer chain degradation was observed
- receiving the so-called cryogel, as a result of photocrosslinking of a mixture of thermoresponsive polyglycidol/photosensitizer after its freezing. These gels were characterized by high mechanical strength, did not degrade during irradiation and, importantly, had a rapid reaction (seconds) to hydration and dehydration under temperature changes
- the thermoresponsive cryogels, obtained in habilitation works, with phase separation temperature equal to 25 °C, were used for the study of their interaction with cells. This choice was justified by the fact that this cryogel is hydrophobic under cell culture conditions (37 °C) and may therefore promote cell adhesion and proliferation.

3.2. Thermoresponsive polymer layers immobilized on a support

As mentioned earlier, in the habilitation work the suitability of thermoresponsive polymer layers based on modified polyglycidol immobilized on a solid support for cell sheet culture and detachment has also been undertaken. Due to the significant progress made in the field of chemistry of temperature-sensitive polymers, these studies have also been extended to other thermoresponsive polymers: poly[oligo(ethylene glycol) methacrylates] and poly(2-substituted-2-oxazolines).

This chapter will first describe the synthesis and properties of layers based on modified polyglycidol immobilized on a support (subsection 3.2.1), followed by layers of poly[oligo(ethylene glycol) methacrylates] (subsection 3.2.2) and poly(2-substituted-2-oxazoline)s (Section 3.2.3). Chapter 3.3 shows the use of these thermoresponsive layers in a cell sheet culture and detachment.

3.2.1. Thermoresponsive polyglycidol immobilized on a support [H5]

Polymer surfaces containing the layer made of thermoresponsive polyglycidol derivative (Fig. 1.2 B) were obtained using a grafting-to technique. The synthesis of thermoresponsive layers was analogous to that described in Section 2.1 for polyglycidol layers tested for protein adhesion. It included two steps: modification of solid support to introduce reactive anhydride groups (Fig. 2.1 A, B and C) and then immobilization of pre-synthesized polymers of glycidol (Fig. 2.2 A), in this case thermoresponsive ones.

Poly(glycidol-co-ethyl glycidyl carbamate) ($M_n = 2 \cdot 10^6$ g/mol, $M_n/M_w = 1.3$), having 40 % of ethyl carbamate groups in the polymer chain, was used for the study. This polymer (mPGl – the polymer designation in the publication) showed a transition temperature at 25 °C. At this temperature the polymer was hydrophobic, which can promote adhesion and cell proliferation.

The above polymer at different concentrations (0.25% to 10%) was applied onto silica or glass substrates containing PE-MA layer. Due to the reaction of anhydride groups with hydroxyl groups derived from mPGl, a polymer layer, in which the polymer chains were repeatedly attached with the support, was obtained.

The presence of the polymer on the substrate was confirmed by FT-IR. A characteristic absorption bands derived from groups present in mPGl have been observed in the spectrum. After grafting reaction, the absorption band at 1850 cm^{-1} derived from the C=O groups of the PE-MA layer disappears. This proves that most of the anhydride groups of the PE-MA layer have reacted with the hydroxyl groups of poly(glycidol-co-ethyl glycidyl carbamate).

An AFM analysis showed that the morphology of the mPGl layers depended on the concentration of the polymer solution used during immobilization. For concentrations of 0.25 % to 1 %, polymer layers with a smooth and regular surface (RMS = 0.09-0.18 nm) were obtained (Fig. 3.6 A). Using a more concentrated solution (10%) resulted in a "wavy" layer of irregular surface (RMS 1.3 nm) (Fig. 3.6 B).

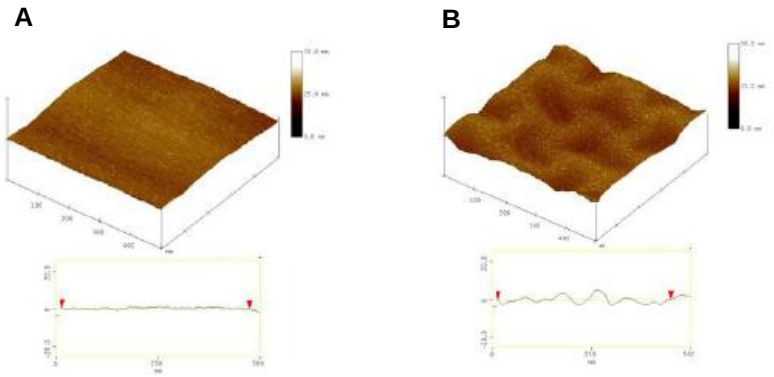


Fig. 3.6. AFM micrographs of mPGl surfaces obtained during immobilization of mPGl with concentrations: (A) 0.25 % and (B) 10 %.

By using ellipsometry, thicknesses of polymer layers were measured whereas contact angle measurements provided information on the affinity for water (Table 2.5). The tests were performed for dry layers and layers incubated in water at 20 °C and 40 °C (below and above the T_{CP} of the polymer immobilized on a support). This allowed to assess whether the obtained biomaterials were thermoresponsive and could be used for cell sheet adhesion, proliferation and separation.

Table 2.5. Thickness of mPGl layers and their affinity for water

mPGl concentration	Contact angle [°]		Layer thickness [nm]		
	water/20 °C	water/40 °C	dry	water/20 °C	water/40 °C
0.25%	60±3	68±2	20±0.7	30±0.5	36±0.5
0.5%	55±3	65±4	28±0.5	31±0.4	33±0.4
1%	50±3	70±2	34±0.5	38±0.4	41±0.5
10%	30±3	55±2	24-60±0.5[a]	27-60±0.5[a]	47±0.5
PGl	50±3	51±3	---[b]		

[a] the layer thickness alter depending on the place of measurement
[b] not determined

The average contact angle of the mPGl layers at 20 °C varied between 30 and 60 °, depending on the concentration of the polymer solution used for the immobilization. At 40 °C, the affinity of the polymer layer for water changed and the contact angle increased. This indicates on an increase in hydrophobicity of the polymer surface after the exceeding of T_{CP} of the immobilized polymer. For comparison, the affinity for water of surfaces coated with unmodified polyglycidol, which is hydrophilic and does not exhibit thermoresponsivity, did not change with increasing temperature.

The thickness of the dry polymer layer increased from 20 nm to 34 nm with the increase of mPGl concentration. For a 10 % polymer solution, an irregular and "wavy" layer of thickness from 24 to 60 nm was obtained (heterogeneity of the layer was also confirmed by AFM, Fig. 3.6 B). The thickness of all layers incubated in water at 20 °C increased, indicating their swelling due to water absorption. Surprisingly, the increase of temperature to 40 °C (that is above T_{CP} of mPGl) resulted in a slight increase in layer thickness. It is known that for layers of polymer brush structures, the thickness decreases with increasing temperature. This is a result of shrinkage of polymer chains [31]. The behavior observed for mPGl layers, and described in [H5], may be the result of the specific polymer layer structure consisting of the interpenetrating polymer chain that are multiply grafted onto the support (Fig. 3.7). A heating of such system cause that, despite dehydration of the layer, the polymer chains are "stretched" due to hydrophobic interactions between them.

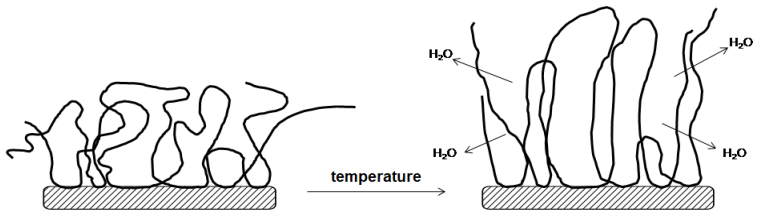


Fig. 3.7. The scheme of the reaction of poly(glycidol-co-ethyl glycidyl carbamate) polymer chains immobilized on a support on the temperature changes.

The surfaces obtained from poly(glycidol-co-ethyl glycidyl carbamate)s were thermoresponsive – they changed their affinity for water as the temperature increased. In the latter part of the habilitation work, they were used for studies related to cell sheet culture and detachment.

3.2.2. Thermoresponsive poly[oligo(ethylene glycol) methacrylates)] on support [H4, H6]

Poly[oligo(ethylene glycol) methacrylates)] (POEGMA), except of the modified polyglycidol, were also used to obtain thermoresponsive polymer coatings. POEGMA is a large group of polymers, which in recent years has gained considerable interest [32]. Most monomers of oligo(ethylene glycol) methacrylates are commercially available and easily polymerizable using controlled radical polymerization techniques (especially ATRP – controlled atom transfer radical polymerization). The amphiphilic structure of these polymers (where the side chain of oligo(ethylene glycol) is responsible for the solubility and formation of hydrogen bonds with water molecules whereas the main chain for competing hydrophobic interactions) cause that many of them exhibit thermoresponsive behavior. Compared to the commonly used PNIPAM, the thermoresponsive POEGMAs have many advantages, for example, they are characterized by a narrow phase transition with a slight hysteresis, and the influence of the external factors on their T_{CP} values is small. **At the time of the habilitation work, the use of poly[oligo(ethylene glycol) methacrylates)] immobilized on the support for cell sheet culture and detachment was not described.**

- The choice of polymer with desired phase transition temperature [H6]

As a part of habilitation work, a number of (co)polymers based on tri(ethylene glycol) monoethyl ether methacrylate (TEGMA-EE) were synthesized using the ATRP technique. As the second comonomer oligo(ethylene glycol) monomethyl methacrylate – $OEGMA_{475}$ was chosen, where 475 is the molar mass of the ethylene glycol units in the mer. The aim of the study was to determine the effect of copolymer composition on copolymer phase transition temperature, followed by the selection of a polymer exhibiting T_{CP} at temperature favorable for cell culture (Fig. 3.8)

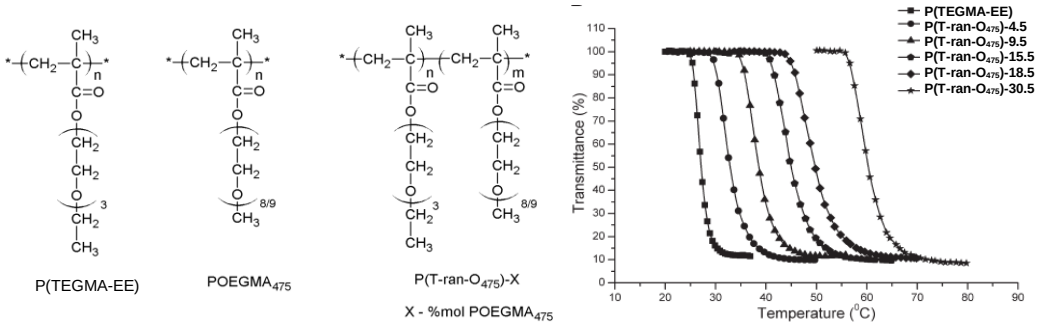


Fig. 3.8. The chemical structure of the synthesized (co)polymers of TEGMA-EE and the figure presenting the dependence of transmittance of their water solutions as a function of temperature (conc. 0.5 g/L).

^{1}H NMR and FT-IR techniques were used to confirm the chemical structure of the (co)polymers and to calculate their composition. The composition of the copolymers ranged from 4.5 mol % to 30.5 mol% $OEGMA_{475}$ and was in each case close to that of the initial reaction mixture. The molar masses, obtained with the use of GPC-MALLS, were in the range of 24 500 g/mol to 50 200 g/mol. Chromatograms of all (co)polymers were monomodal and symmetrical and M_w/M_n did not exceed 1.34. The (co)polymers with phase transition temperature of 25-60 °C, dependent on the composition and the molar mass, were obtained (Fig. 3.8).

For the synthesis of polymer surface poly[tri(ethylene glycol monoethyl ether) methacrylate] (P(TEGMA-EE)), whose T_{CP} was 25 °C (5 g/L) was chosen.

➢ Immobilization of poly[tri(ethylene glycol monoethyl ether) methacrylate] on support [H4]

The poly[tri(ethylene glycol monoethyl ether) methacrylate] surfaces were obtained using grafting-from technique [H4], where bromide groups immobilized on silica or glass substrate initiated the atom transfer radical polymerization (SI-ATRP) of TEGMA-EE. For the synthesis of Si~P(TEGMA-EE) polymer layer four-step procedure composed of hydroxylation of substrates, their aminosilylation, introduction of ATRP initiator and SI-ATRP of TEGMA-EE was used (Fig. 3.9).

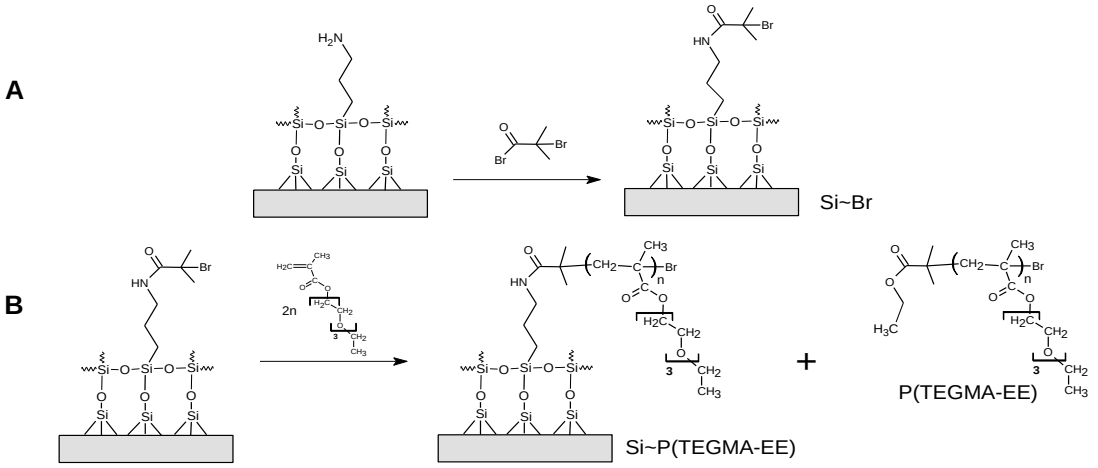


Fig. 3.9. The scheme of the synthesis of Si~P(TEGMA-EE) layers: (A) immobilization of ATRP initiator and (B) SI-ATRP of tri(ethylene glycol monoethyl ether) methacrylate.

The hydroxylation and aminosilylation step (introduction of amino groups derived from APTES) were analogous to those described in section 2.1 (Fig. 2.1 B). The SI-ATRP initiator, formed during the reaction of amino groups with 2-bromo-2-methylpropionyl bromide (Si-Br), subsequently initiated the polymerization of the TEGMA-EE monomer. A surface with a polymer brush structure was obtained (Si-P(TEGMA-EE), Fig. 1.2 B II).

It is known [33], that in the case of ATRP initiated from the substrates low concentration of the initiator groups located only on the substrate leads to uncontrolled polymer chain growth. In order to ensure the control over polymerization an initiator non-bound with the substrate ("sacrificial initiator") was introduced into the system. The polymerization occurs then simultaneously in solution and on the substrate. The molar mass of the polymer that is formed in solution can thus be referred to the molar mass of the polymer grafted from a support [33].

The molar mass of the polymers formed in the solution increased from 23 000 to 189 000 g/mol with the polymerization time. Chromatograms of all P(TEGMA-EE) samples were monomodal and narrow. The molar mass dispersity for polymers obtained within the first 6 hours of polymerization was lower than 1.3 and then increased to 2.3.

XPS analysis of surface compositions confirmed the aminosilylation reaction and the introduction onto a substrate the ATRP initiator. This is indicated by an increase in the nitrogen and carbon content followed by the appearance of bromine atoms (Table 2.6). The formation of the Si~P(TEGMA-EE) layer led to the attenuation of the nitrogen signals, while the intensity of the silica signal decreased significantly. The intensity ratio of the C/O signals was increased from 0.5 (for Si~Br) to 2.77 (for Si-P(TEGMA-EE)-21h). The theoretical C/O ratio calculated for tri(ethylene glycol monoethyl ether) methacrylate is 2.4, which well correlates with the measured value.

Table 2.6. Composition of modified substrate and substrate coated with polymer P(TEGMA-EE)

Designation of the layer in publication	C1s	O1s	Si2p	N1p	Br
Si~OH	17.2	57.4	24.7	0.7	0.0
Si~NH_2	28.8	47.7	20.8	2.7	0.0
Si~Br	25.9	51.3	20.5	2.2	0.1
Si~P(TEGMA-EE)	72.5	26.2	1.23	0.0	0.0

- Analysis of Si~P(TEGMA-EE) layer properties [H4]

The roughness of the Si~P(TEGMA-EE) layers changed with the polymerization time, which was related to the increase of polymer chains. RMS increased from an initial value of 0.24 nm (after one hour of polymerization) to a maximum of 0.62 nm for the layer after 4 hours of polymerization. A further increase in the polymerization time resulted in the formation of a homogeneous film (due to the elongation of the polymer chains), thereby decreasing the RMS to 0.21 nm was observed.

The thickness of the Si~P(TEGMA-EE) layer and its affinity for water was measured for dry layers and incubated in water at 20 °C (<T_{CP}) and 37 °C (> T_{CP}) (Fig. 3.10).

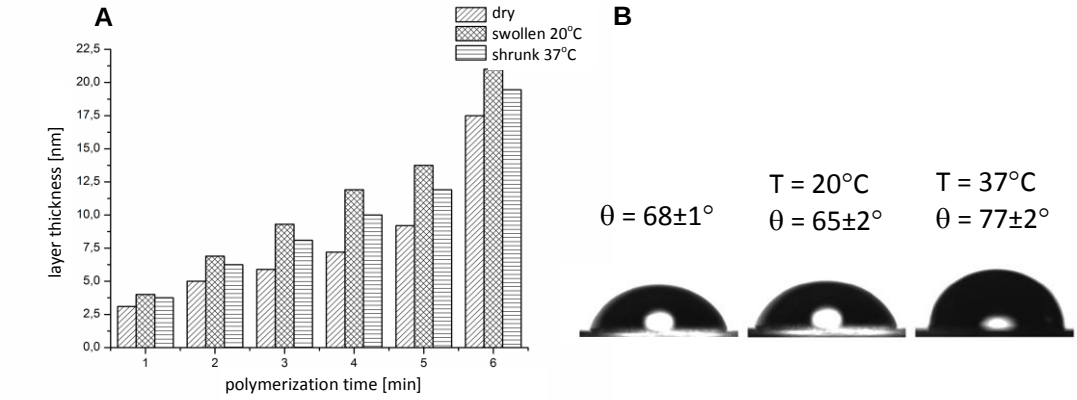


Fig. 3.10. (A) Thickness of Si~P(TEGMA-EE) layers in the dry state and after incubation in water at 20 °C and 37 °C as a function of polymerization time; (B) Photos of water drops and contact angle for Si~P(TEGMA-EE) layers under different conditions.

The thickness of the polymer layer in the dry state increased with the polymerization reaction time from 3 nm to 18 nm (Fig. 3.10 A). A significant increase was observed during the first 6 hours of polymerization. Further prolongation of the polymerization time caused a slowdown in layer thickness increase due to reduction in monomer concentration in the reaction system, loss of active centers or reduced monomer diffusion rate. Incubation of the surfaces in water at 20 °C caused their swelling and the layer thickness increase. When the temperature was raised to 37 °C a decrease in layer thickness due to the shrinking of the polymer chains, caused by the weakening of hydrogen bonds between the water molecules and the polymer, was observed. Dehydration of the polymer coating also has a reflection in the larger contact angle values (Fig. 3.10 B). The Si~P(TEGMA-EE) coating changed its affinity for water with the temperature change.

Changes in layer thickness and contact angles due to temperature increase indicated that the Si~P(TEGMA-EE) layers were thermoresponsive. That is why they were applied for cell sheet culture and detachment.

3.2.3. Thermoresponsive poly(2-substituted-2-oxazoline) on a support [H7, H8, H9]

Another group of thermoresponsive polymers used for the investigation of their interaction with cells were poly(2-substituted-2-oxazoline)s (POx).

POx, called pseudopeptides, are non-toxic and biocompatible [34] and do not accumulate in tissues [35]. Although the polymerization mechanism and the 2-oxazoline polymers themselves have been known for several decades, due to their interesting properties these polymer were discovered again. It has been shown that it is possible to obtain POx copolymers with different side chains or end groups and different architectures [36]. There are numerous applications of POx in medicine and biotechnology, such as POx-protein conjugates or DNA carriers [37]. Some of POx are thermoresponsive, which additionally enhances their attractiveness for biomedical applications.

At the time of habilitation work, there was few work concerning POx immobilization on a support [38]. None of them have described the preparation of poly(2-substituted-2-oxazoline) layers with a structure of polymer brush using the termination of living POx chains by a functional groups of the support. They also did not refer to the thermoresponsive behavior of the polymeric coatings, also in terms of their interaction with cells.

- Immobilization of POx on a support [H7, H8]

Copolymers of 2-ethyl- and 2-nonyl-2-oxazoline (PENOx) and homopolymer of 2-isopropyl-2-oxazoline (PIPOx) were chosen for the studies.

Polymer surfaces containing the thermoresponsive POx were obtained by using a grafting-to technique [H7, H8]. For this purpose, living cationic chain ends of the PENOx copolymer or PIPOx homopolymers were terminated by the amino groups of the substrate (derived from APTES). A covalent bonds were formed and surfaces with a polymer brush structures were obtained (Fig. 1.2 B_II and Fig. 3.11).

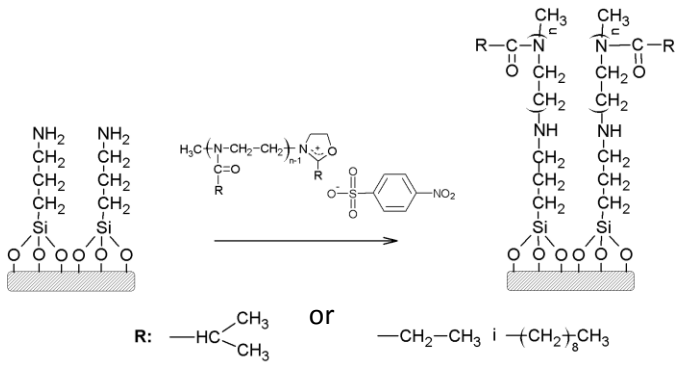


Fig. 3.11. The general scheme of the (co)poly(2-substituted-2-oxazoline)s surface synthesis.

The properties of the 2-substituted-2-oxazoline (co)polymers that were grafted onto the support are shown in Table 2.7.

Table 2.7. Characteristic of (co)poly(2-substituted-2-oxazoline)s in solution, subsequently grafted to the support [H7,H8]

Polymer/layer designation in publication	M_n (SEC-MALLS)	M_w/M_n	mol% of NOx (NMR)	T_{CP} [°C]	t_{POL} [days][a]
sPIPOx0	20800	1.01	---	37	0
sPIPOx2					2
sPIPOx4					4
sPIPOx7					7
PIPOx1	22500	1.02	---	37	12
PIPOx2	40000	1.06	---	35	12
PENOx1	15000	1.04	14	22	12
PENOx2	18000	1.27	14	22	
PENOx3	14000	1.27	10	31	12
PENOx4	21800	1.30	10	31	
PENOx5	15300	1.09	6	36	12

[a] The time after which the polymer has been grafted to the substrate after reaching 100% conversion

The living (co)polymers PIPOx and PENOx were immobilized to the substrates at different times after reaching the 100% conversion (t_{POL} from 0 to 12 days, Table 2.7).

During AFM analysis, it was observed that fibrillary structures were formed on PIPOx layers. The presence of these structures resulted from the partial crystallization of poly(2-isopropyl-2-oxazoline) in acetonitrile, the solvent used for polymerization. **Because the observed phenomenon has not been explained so far, in habilitation work [H9] the study of the crystallization process of PIPOx in acetonitrile has been undertaken.**

- POx crystallization in organic solvents [H9]

The crystallization of poly(2-substituted-2-oxazolin) in aqueous solutions and in aqueous solutions with addition of organic solvents has been described in the literature [39,40]. The crystallization of POx in these solvents is induced by prolonged heating of the solution above T_{CP}. The principal role in the crystallization mechanism has been thus attributed to the dehydration of polymer chains during phase transition and the formation of so-called "polymer rich phase" [39].

The crystallization of poly(2-isopropyl-2-oxazoline) observed in the work [H7, H8] occurred in an organic solvent, in which no phase separation of the polymer is observed. This seems to contradict the above interpretation, according to which the presence of water is necessary for the POx crystallization process. Therefore, in work [H9] the crystallization of PIPOx in acetonitrile (ACN), dimethyl sulfoxide (DMSO) and propylene carbonate (PC) was investigated. In the dissertation, the results of crystallization of PIPOx only in ACN were shown, as this mixture was used to immobilize the polymer on substrates, and obtained layer was used for interaction with cells.

PIPOx solutions (M_n = 20 800 g/mol, M_w/M_n = 1.01) in acetonitrile (5 %, 10 % and 30 %) were heated at 50 °C for 20 days. At this time, the mixture became cloudy and a precipitate was formed. The precipitate was analyzed by DSC and WAXS (Fig. 3.12).

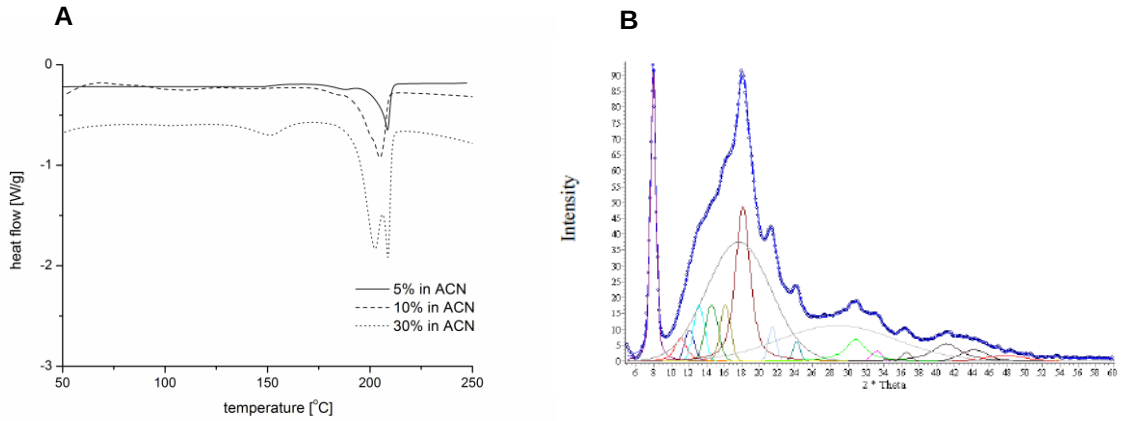


Fig. 3.12. (A) DSC traces for PIPOx crystallized from ACN at different concentration (heating rate 10 °C/min) and (B) X-ray diffraction curves for precipitate derived from 5 % solution of PIPOx in ACN.

The DSC thermograms of precipitates formed in all PIPOx solutions showed the presence of an endothermal peak indicating that the samples contained a crystalline fractions that melted. The enthalpy of melting increased with increasing the concentration of the solution from which the precipitate was formed (from 20 J/g for 5 % solution to 41 J/g for 30 % solution). WAXS analysis revealed two principal diffraction peaks at 2θ = 7.93 ° and 18.11 °. Identical peak positions and intensities were obtained for PIPOx crystallized in water [41]. This means that PIPOx crystallized in organic solvents has the same elemental cell as PIPOx crystallized in water. The highest crystal fraction χ_c of 68 % was obtained for PIPOx crystallized from a 30 % solution in ACN.

The morphology of PIPOx crystallized from organic solvent was analyzed by SEM (Fig. 3.13).

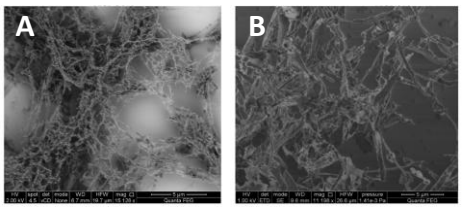


Fig. 3.13. SEM micrographs of PIPOx crystallized from ACN of 30 % (A) and 5% (B) (scale 5 µm).

In the SEM micrograph of PIPOx, a network-like structure is present. Within this network, separate objects possessing a fibril-like morphology can be distinguished (Fig. 3.13). The length of the fibrils ranged from a few to several microns, with a width of approximately 50 nm. It was shown that the concentration of the solution from which PIPOx crystallized, had little influence on the morphology of the resulting structures.

The studies demonstrated that PIPOx crystallize in an organic solvent (without water) and this process is not related to the thermoresponsivity of the polymer.

➢ Analysis of the properties of poly(2-substituted-2-oxazoline) layers [H7, H8]

Due to the observed PIPOx crystallization in the polymerization solvent [H9], its immobilization on the support was performed immediately after the monomer was fully converted (sPIPOx0) and after two (sPIPOx2), four (sPIPOx4), seven (sPIPOx7) and 12 (PIPOx1) days of annealing of the polymerization solution (Table 2.7). This enabled us to determine the effect of the amount of crystallites on the surface properties, including the thermoresponsive behavior of the surfaces and their ability to interact with the cells. PENOx copolymers did not crystallize from the solution thus they were immobilized 12 days after reaching full conversion.

The morphologies of the obtained polymer layers are shown in Figure 3.14.

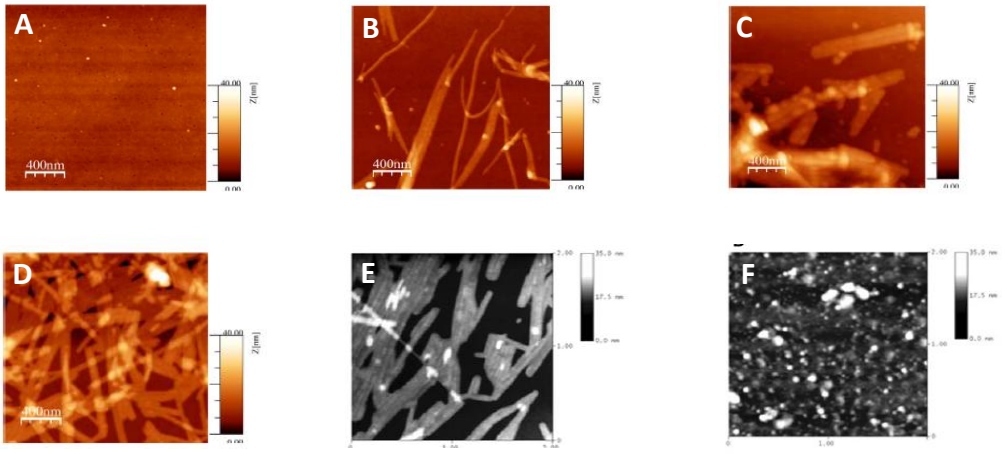


Fig. 3.14. AFM micrographs of sPIPOx0 (A), sPIPOx2 (B), sPIPOx4 (C), sPIPOx 7 (D), PIPOx2 (E) and PENOx1 (F) layers.

When the PIPOx was grafted to the substrate immediately after reaching full conversion, the polymer surfaces were smooth, without fibril structures and the RMS was low (0.4 nm) (Fig. 3.14 A). When the time, after which the polymer has been tethered on the surface, was elongated the amount of fibrils in the surface was increased (from 20 % to 70 %, Fig. 3.14 B-E). The surfaces were more rough and inhomogeneous (RMS up to 6-12 nm). At the same time, a layer thickness increase from 5 nm to 11 nm was observed. The length of fibrils deposited on the surface reached several microns.

To remove crystallites from the polymer layer, the surfaces were heated at 210 °C (above T_m of PIPOx) and then quenched and washed. The resulting layer was homogeneous and smooth (RMS = 0.5 nm) and its thickness (5 nm) corresponded to the thickness of the layer obtained during immobilization of sPIPOx0 to the substrate. The results indicated that crystallites were only embedded on the amorphous PIPOx layer, not covalently bonded, and could be easily removed.

The morphology of the surfaces based on copolymers of 2-ethyl- and 2-nonyl-2-oxazolines (PENOx) was rough and nonhomogeneous (Fig. 3.14 F). Although the 2-nonyl-2-oxazoline homopolymer is a crystalline polymer [42], its copolymers with 2-ethyl-2-oxazoline are amorphous. Therefore, the presence of fibrils was not observed on the surface of PENOx. The surface morphology of PENOx depended on the copolymer composition. The lower the amount of NOx in the copolymer the more regular surface morphology and lower surface roughness (RMS drops from 1.1 nm to 0.25 nm for 14 % and 6 % of NOx, respectively).

Water affinity and thickness of PENOx and PIPOx layers were investigated in the study to evaluate the effect of molar masses, copolymer compositions, or the presence of crystallites (non-thermoresponsive) on the behavior of the layers incubated in water at different temperatures (Table 2.8).

Table 2.8. Thicknesses of PENOx i PIPOx layers and their affinity for water in dry state and after incubation in water at 20 °C and 40 °C

Designation of polymer/layer in publication	Crystallite content [%] *or* % NOx	Contact angle [°]			Thickness [nm]		
		Θ_1 dry layer	Θ_2 water 20 °C	Θ_3 water 40 °C	h_1 dry layer	h_2 water 20 °C	h_3 water 40 °C
sPIPOx0	0	65	54	64	5	12	7
sPIPOx2	20	68	58	67	6	12	7
sPIPOx4	40	69	58	68	7	13	9
sPIPOx7	70	73	60	64	9	13	11
PIPOx1	70	77	62	75	9	13.5	12
PIPOx2	70	72	60	70	11	16	13
PENOx1	14	78	55	70	5	7	6.5
PENOx2	14	71	60	68	6	9	7
PENOx3	10	67	58	65	4	5.5	4
PENOx4	10	57	50	56	8	12	9
PENOx5	6	-	-	-	5	8	6.5

It was observed that the contact angle of PENOx dry surfaces depended on the composition and molar mass of the immobilized copolymer. The NOx comonomer is hydrophobic and its greater amount in the chain caused the layer to have less affinity for water. For layers with the same NOx content, the layer was more hydrophilic when the molar mass of the immobilized polymer was higher. The affinity for water of the PIPOx layers depended on the crystallites content on the surface and decreases with the increase in the number of fibrils on the surface, confirming their hydrophobic character. Contact angles of all the surfaces obtained after incubation in water at 20 °C decreased in comparison to dry layers. This was due to the solvation of polymer chains under the penetration of water into the layer.

The thicknesses of the obtained POx layers were in the range of 4 to 11 nm and were depended on the molar mass of the immobilized polymer and on the number of crystallites on the substrate. Incubation of polymer surfaces in water at 20 °C caused swelling of polymer layers – an increase in layer thickness was observed. For PIPOx layers, the increase in layer thickness (h2-h1, table 2.8) was depended upon the number of crystallites on the surface. Hydrophobic crystallites on the surface prevent from water penetration into the polymer layer and therefore limit its ability to swell.

At a temperature of 40 °C, above the T_{CP} of immobilized POx, the layers became hydrophobic. The simultaneous decrease in layer thickness, almost to its initial value, was observed. This demonstrates the dehydration and shrinkage of polymer chains grafted onto the substrate as a result

of the disorder of the solvation sphere. The response of the sPIPOx7 layer, that contains 70 % of the crystallites on the surface, to the temperature change (Θ_3-Θ_2 = 4) was the smallest.

The temperature-induced swelling (below T_{CP}) and shrinking (above T_{CP}) of (co)poly(2-substituted-2-oxazolins) layers, obtained in the work, with simultaneous changes in their water affinity are characteristic for thermoresponsive layers. Therefore, these surfaces, in the subsequent stages of the studies, were used for the adhesion and detachment of the cells in a form of a sheet.

Within the works presented in articles [H4-H9]:

- polymer layers based on (co)polymers of glycidol and (co)polymers of 2-substituted-2-oxazolines were obtained, using grafting-to methods, and characterized. In the first case, a layers with the structure of interpenetrating chains multiply attached to the substrate were obtained, whereas in a second case a layers of the polymer brush structure were prepared,
- surfaces based on the tri(ethylene glycol) monoethyl ether methacrylate with a polymer brush structure were obtained via grafting-from methods,
- it was established that the mPGl, P (TEGMA-EE), PIPOx and PENOx-based layers exhibited thermoresponsive behavior – their thicknesses and affinity for water changed with changes of the environmental temperature. Therefore, these layers were used for their interaction with cells.

3.3. Thermoresponsive polymer layers in a culture and detachment of skin cell in a form of a sheet [H1, H4, H5, H7, H8, H11]

The ability of the thermoresponsive layers, based on polyglycidol (both self-supporting and immobilized on the support), poly[tri(ethylene glycol monoethyl ether) methacrylate] and poly(2-substituted-2-oxazolines) to interact with cells, particularly to culture a continuous cell sheet and then detach it without using enzymatic methods of cell separation, was investigated.

Skin cells (fibroblasts and keratinocytes) were used in the studies, which obtained in a form of a sheet may be applied to wounds caused by burns or chronic diseases. Prolonged healing of wounds and difficult methods of supplying them are a great challenge for modern medicine. Modern biological and chemical dressings unfortunately do not guarantee effective treatment due to the possibility of rejection, the formation of unwanted scars or the possibility of transfer of pathogens. A methods of tissue engineering provide a variety of possibilities for the use of cell and tissue cultures for the treatment of many diseases including burns and chronic wounds. Studies on the populating of scaffolds with patient's own cells or using the cells as a suspension directly to the wound prove the limited applicability of such methods. The use of thermoresponsive surfaces to obtain a prototype of a tissue, e.g. the epidermis produced from the patient's own cells, seems to be an interesting solution.

Studies of skin cell culture and detachment were conducted in cooperation with the Department of General, Molecular Biology and Genetics, Medical University of Silesia and the Center for Burns Treatment in Siemianowice Slaskie.

3.3.1. A skin cell sheet culture [H1, H4, H5, H7, H8]

- Skin cell adhesion and proliferation

The studies were conducted using both self-supporting polymer layers based on modified polyglycidol and layers based on polymer of glycidol immobilized on a support, poly[tri(ethylene glycol monoethyl ether) methacrylate] and poly(2-substituted-2-oxazolines) layers. Skin cells were seeded on all tested polymer layers at 37 °C. At that temperature, well above the T_{CP} of tested polymers, the layers as shown in the work were hydrophobic. Cell viability was measured after certain hours of cell culture using the AlamarBlue test. TCPS, a standard surface for cell culture, was used as the control surface. It was observed that in the studied conditions the properties of all the obtained layers are favorable to cell adhesion and proliferation (exemplary graph is shown for the Si~P(TEGMA-EE) layer in Fig. 3.15 A).

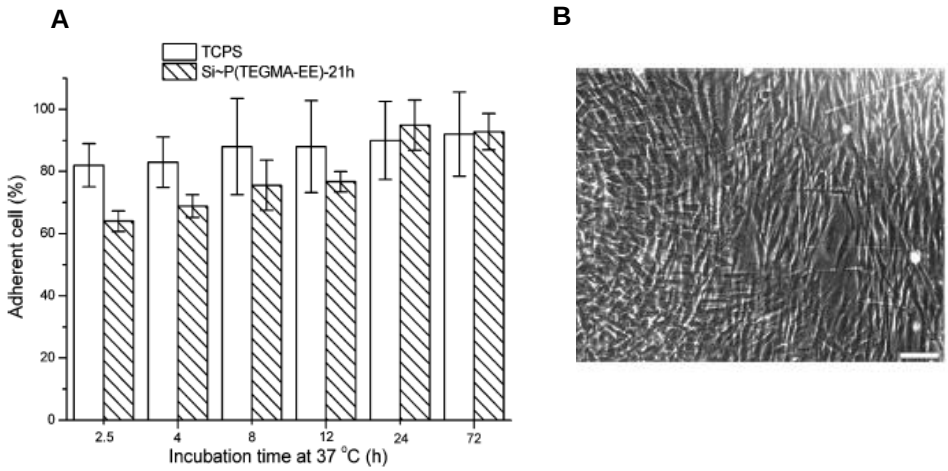


Fig. 3.15. (A) Percentage of cells that adhered and proliferated on the Si~P(TEGMA-EE) layers compared to the TCPS control surface; (B) Morphology of fibroblasts grown in a form of sheet on Si~P(TEGMA-EE) surface after 24 h of culture at 37 °C (100 μm scale).

After 24 hours of culture, 60 to 100% of the cells (depending on the type of tested layer) were adhered on the thermoresponsive layers compared to the control surface. After certain time, the cells were proliferated on each of the polymer layers and formed an integrated sheet within which they were closely packed and connected by the extracellular matrix (Fig. 3.15 B) [H1, H4, H5, H7, H8].

It was established that 60% of fibroblast cells adhered to self-supporting layers (cryogels) based on modified polyglycidol [H1]. In the case of polymer layers, based on modified polyglycidol immobilized on a substrate, the number of cells that have adhered depended on the polymer layer thickness. In addition, the thickness of the layer has a significant effect on the homogeneity of the resulting cell sheet [H5]. On the surface with the greatest thickness the cells formed a discontinuous sheet. Both cell-free places and clusters of cells were observed. This was probably due to the heterogeneity of the polymer layer (Fig. 3.6 B, Section 3.2.1). Fibroblasts accumulated in the hollows of the polymer layer, where the layer was thinner. The keratinocytes, in comparison to fibroblasts had lower tendency to adhere on a mPGl surfaces. The addition of laminin (the main component of the intracellular matrix) to the culture medium increased the adhesion of keratinocyte and allowed them to proliferate.

For Si~P(TEGMA-EE) surfaces, it was observed that the layer thickness had no significant effect on the degree of fibroblast adhesion [H4]. After 24 hours of culture, almost 100 % of the cells were attached to each of the tested Si~P(TEGMA-EE) layers. After this time, the cells grew and formed a continuous sheet.

For PIPOx-based layers, the number of cells that have adhered and proliferated increased with the increase of the amount of crystallites and with the layer roughness (Fig. 3.14, Section 3.2.3) [H8]. The number of cells cultured after 24 hours on the sPIPOx0 (0% crystalline) layer increased at least one and a half times compared to the number of cells at the beginning of the culture. Whereas for the sPIPOx7 layer (70% of crystallites), the number of cells tripled. It appears that hydrophobic, crystalline PIPOx fibrils embedded on the surface resemble the arrangement and size of fibrous proteins (such as collagen, elastin or fibronectin) in the extracellular matrix, which may promote cell adhesion. In addition, the fiber-mesh of PIPOx crystallites may facilitate the circulation of fluids that supply oxygen and nutrients to cells as well as the flow of metabolites (including waste) from cells to the ECM, making the culture more efficient. It was further established that the degree of fibroblast adhesion to PIPOx surfaces was greater than that for PENOx surfaces [H7]. In the case of PENOx surfaces, neither molar mass nor hydrophobic content of nonylooxazoline had significant effect on cell adhesion. After 24 hours of culture, the fibroblasts on all PENOx layers adhered to the same extent as in the control sample.

- The skin cell sheet detachment

After formation of a continuous sheet, the temperature of the culture was lowered below the T_{CP} of the immobilized polymer (to 20 °C or 17.5 °C depending on the surface). As shown in the studies conducted in the habilitation work, all obtained polymer layers changed their properties in these conditions: they became hydrophilic and the layer thickness changed (Fig. 3.5 and 3.10 and Table 2.5 and 2.8). The changing of the layer properties should promote the spontaneous separation of the cells in the form of a sheet.

For any layer of the thermoresponsive polyglycidol (either self-supporting or immobilized on the substrate) there was no satisfactory effect of the detachment of the fibroblast sheet after reducing the culture temperature to 20 °C [H1, H5]. In the case of a self-supporting layer, its porous structure, which, along with its hydrophobicity, favored cell culture, probably led to the "trapping" of cells during proliferation. This prevented the sheet from being detached when the properties of the layer has changed at reduced temperature. In the case of layers made of thermoresponsive polyglycidol immobilized on the surface, the unusual layer structure (the penetrating chains that are repeatedly grafted to the support) is likely to be responsible for that situation. Although, after the reduction of the culture temperature below T_{CP} the layers became hydrated and hydrophilic, their thickness decreased (Fig. 3.7, Table 2.5). Under the influence of such unusual changes, the cytoskeletal tensile forces of the cells were probably not sufficient to induce a cell shape change from spindle-like to spherical, thereby releasing the fibroblast sheet from the substrate.

In the case of Si~P(TEGMA-EE) and PIPOx and PENOx surfaces, the reduction of the culture temperature induced the spontaneous detachment of the cell sheet (Fig. 3.16).

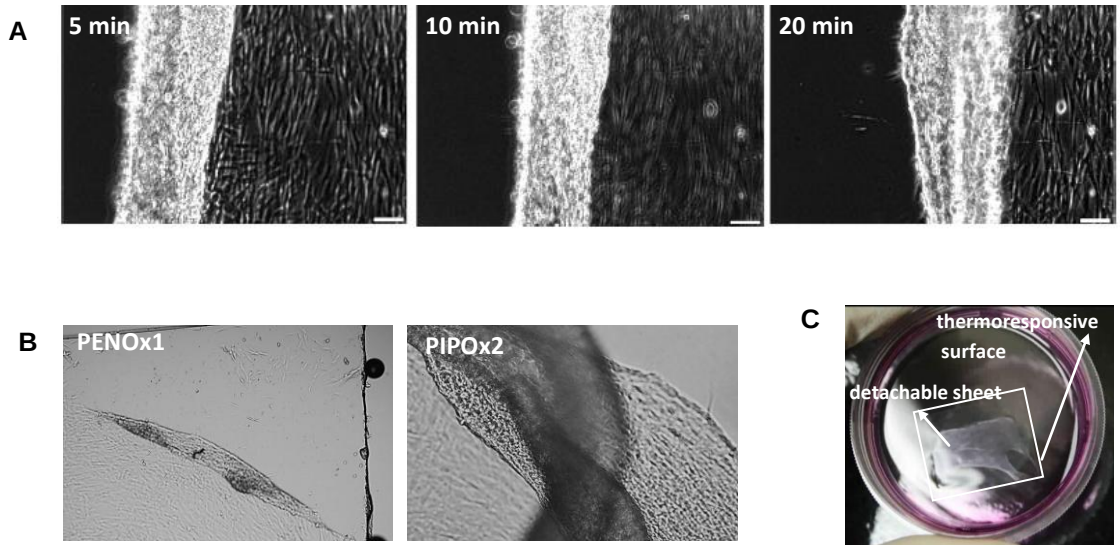


Fig. 3.16. (A) The detachment of fibroblast sheet from Si~P(TEGMA-EE) surfaces after a certain incubation time at 17.5 °C (100 µm scale); (B) and (C) the detachment of fibroblast sheet from poly(2-substituted-2-oxazolin) layers after a certain incubation time at 20 °C.

It was established that in the case of Si~P(TEGMA-EE) layers, the optimum temperature required to detach a sheet of cells is 17.5 °C [H4]. Tests performed in the range of 18-20 °C showed that the cell sheet under these conditions disassembled into pieces or did not detach at all. At 17.5 °C, the fibroblast sheet was completely detached within 40-60 minutes. The sheet detached faster for thicker layers.

In the case of (co)poly(2-substituted-2-oxazolin) layers, the ability of the cells to spontaneous detachment, after lowering the temperature to 20 °C, depends on the composition of the copolymer and on the number of crystallites on the surface [H7, H8]. For PENOx layers, full fibroblast detachment was observed only in the case of PENOx1. This layer was characterized by the most rough surface (RMS about 1 nm) and the largest difference in contact angles between the hydrated and dehydrated layer (Θ_3-Θ_2, Table 2.8). For other PENOx layers, the cells were removed from the substrate only in individual places. The PIPOx layers appeared to be also effective in the detachment of a cell sheet. The ability to detach depends however on the number of crystallites on the surface – the less crystals, the more cells are detached. The higher crystallite content weakens the PIPOx layer's response to temperature change, what was confirmed also by the layer thickness and water affinity tests (Table 2.8), and such behavior has the effect on the ability of the fibroblast sheet for spontaneous detachment. After lowering the temperature to 20 °C, a continuous fibroblast sheet was detached from the PIPOx2 substrate within 30 minutes.

3.3.2. Transfer of fibroblast sheets with membranes [H8,H11]

The sheet of cells rolled up during the detachment from the polymer surface. In order to prevent such situation and to transfer the cell sheet grown on the thermoresponsive surface to the desired place, the possibility of transferring the sheet with the appropriate membrane has been investigated [H8, H11]. SURPATHEL® and Biobrane membranes were tested. Si~P(TEGMA-EE), sPIPOx0 and sPIPOx7 thermoresponsive surfaces were used.

For this purpose, after culturing of the cells on a thermoresponsive polymer surface and obtaining a full confluence, the cells were covered with a SURPATHEL® or Biobrane transfer membrane.

Cells adhered to the membrane. Subsequently, the culture temperature was reduced, which caused the cells to spontaneously detach from the thermoresponsive surface and, together with the membrane, were "raised" from the culture surface. Due to the fact that the cells were attached to the membrane, it was possible to transfer the sheet without rolling it to the new culture surface (Fig. 3.17 shows, for example, the transfer of cells from the sPIPOx0 surface).

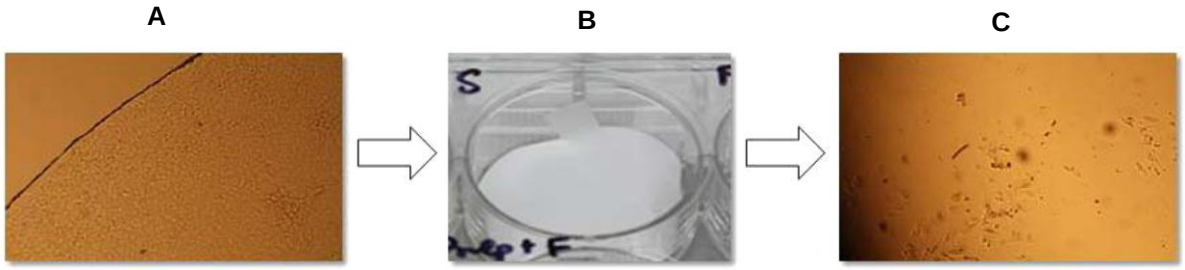


Fig. 3.17. Detachment and transfer of the cell sheet from the sPIPOx0 thermoresponsive surface: (A) fibroblast sheet; (B) coverage of cells with SURPATHEL® membrane; (C) polymer layer after removal of the cell sheet (no visible cell sheet).

It was established, that the SURPATHEL® membrane, as compared to the Biobrane membrane, more efficiently transferred the cell sheet. Almost 90 % of the cell sheet was transferred from the sPIPOx0 surface and 60 % from the sPIPOx7 surface. The sPIPOx7 surface was covered in 70 % of crystallites, thus it was characterized by poor response of physicochemical properties to temperature change. This resulted in lower cell transfer efficiency. In the case of Si~P(TEGMA-EE), cell sheet transfer efficiency was 92 %. It has been shown that cells after transplantation into a new culture surface retain their viability and are capable for further cell division and proliferation.

A similar experiment was conducted for cells grown on TCPS (standard, non-thermoresponsive culture surface). In this case, the cells were not separated from the surface and it was not possible to transfer the sheet. This confirmed the fact that the thermoresponsive properties of the obtained within habilitation work surfaces are necessary detachment of the cell sheet in an intact form.

3.3.3. Biological characterization of skin cells after culture on thermoresponsive surfaces [H7]

Having in mind the usefulness of the obtained polymer surfaces in biomedicine, a researches considering the biological properties of the cells after culture on thermoresponsive surfaces were performed. Cell phenotype, cell gene expression and a genotoxicity of the poly(2-substituted-2-oxazoline) layer was monitored. The results showed that the cells did not change their phenotype during the culture, no damage to the DNA of the cells (Fig. 3.18) or significant changes in their gene expression were detected [H7].

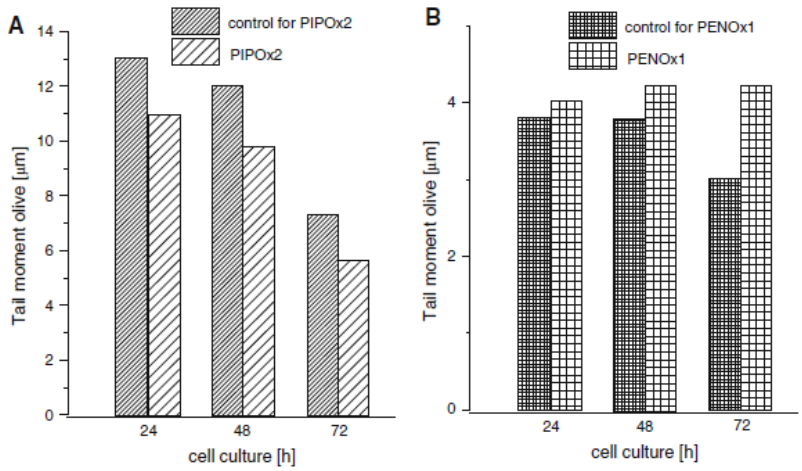


Fig. 3.18. Results of genotoxicity tests based on the comet test for fibroblasts cultured on (A) PIPOx2 and (B) PENOx1. TCPS was used as a control surface.

Based on the results obtained, it can be stated that the thermoresponsive POx surfaces are fully biocompatible and therefore suitable for culturing and detachment of fibroblasts as a continuous cell sheet.

Within the works [H1, H4, H5, H7, H8, H11] it has been shown that:

thermoresponsive Si~P(TEGMA-EE) surfaces of a certain polymer layer thickness and (co)poly(2-substituted-2-oxazoline) surface with a certain crystallite content are suitable for culture and non-invasive cell sheet detachment. By using the SURPATHEL® membrane it is possible to transfer the cultured sheet to a destination place and this treatment does not affect the viability of the cells. It has been also shown that the tested thermoresponsive surface are biocompatible and do not destruct cultured cells, making them attractive for potential use in regenerative medicine.

The obtained results allowed to perform a preliminary medical experiment (data not published), where the patient's cells were grown on the thermoresponsive layer of prepared surface. Then the cell sheet with the membrane was transferred to the wound of the patient. Over the next few days, wound healing was observed.

4. SUMMARY OF A SERIES OF PUBLICATIONS „BIOCOMPATIBLE POLYMER LAYERS OF CONTROLLED AFFINITY FOR WATER. SYNTHESIS AND APPLICATION”

The main purpose of the work was the development and characterization of biocompatible polymer layers for use in regenerative and reconstructive medicine.

A method for the preparation of hydrophilic polyglycidol-based layers with protein-repellant properties has been developed. Various types of layers with different composition and structures (interpenetrating chains multiply grafted with substrate or polymer brushes) were obtained. It has been determined how the composition and structure of the polymer affect the properties of the resulting polymer layers, mainly on the affinity for water, and thus on the reduction of protein adsorption. Based on the obtained results, the possibility to use the obtained biocompatible hydrophilic polyglycidol layers in reconstructive medicine has been presented.

In this work, methods for obtaining thermoresponsive polymer layers based on polymers of glycidol, oligo(ethylene glycol) methacrylates and oxazolines, and their interaction with cells have been developed. It was established how the composition and structure of the polymer affect the properties of the resulting polymeric layers, primarily on the affinity for water both at ambient temperature and at elevated temperature. The layers are hydrophobic at a temperature above the transition temperature of the immobilized polymer. These conditions promote skin cell culture. Lowering the culture temperature below the transition temperature of the immobilized polymer alters the properties of the substrate and thus allows the cells to be detached in form of a sheet. The properties of the obtained layers were correlated with their effectiveness on cell growth and sheet formation, and thus the possibilities of using so obtained surfaces in tissue engineering were presented.

The gained knowledge allowed to present the possibility of using the obtained materials in biomedicine: to reduce protein adsorption and to culture and detach cell sheets.

8 LITERATURA

1. red. M. Olszówka, K. Maciąg „Nowoczesne trendy w medycynie" Lublin **2015**, wyd. fundacja na rzecz promocji nauki i rozwoju TYGIEL.
2. red. B. Ratner, A. S. Hoffman, F. J. Schoen, J.E. Lemons "Biomaterials science" USA **1990**, wyd. Academic Press.
3. V. K. Vendra, L. Wu, S. Krishan "Polymer thin films for biomedical application" w "Nanomaterials for the Life Sciences Vol.5: Nanostructured Thin Films and Surfaces" **2010**, wyd. Challa S. S. R. Kumar WILEY-VCH.
4. J. R. Smith, D. A. Lamprou Transactions of the IMF The International Journal of Surface Engineering and Coatings **2014**, 92(1), 9-19.
5. A. Dworak, B. Trzebicka, W. Wałach, Macromol. Chem. Phys. **1995**, 196, 1963-1970.
6. W. Wałach, A. Kowalczuk, B. Trzebicka, A. Dworak, Macromol. Rapid Commun. **2001**, 22, 1272-1277.
7. P.Y. Yeh, R. Kainthan, Y. Zou, M. Chiao, J. Kizhakkedathu, Langmuir **2008**, 24, 4907-4916.
8. M. Wyszogrodzka, R. Haag, Langmuir **2009**, 25, 5703-5712.
9. M. Wyszogrodzka, R. Haag, Biomacromolecules **2009**, 10, 1043-1054.
10. M. Jamróz-Piegza, A. Utrata-Wesołek, B. Trzebicka, A. Dworak, Eur. Polymer J. **2006**, 42, 2497-2506.
11. Z. Tang, Y. Akiyama, T. Okano, Polymers **2012**, 4, 1478-1498.
12. M. Keerl, V. Simirnovas, R. Winter, W. Richtering, Angew. Chem. **2008**, 120, 344-347.
13. B. D. Ratner, J. Dent. Educ. **2001**, 65, 1340-1347.
14. S. R. Meyers, M. W. Grinstaff, Chem. Rev. **2012**, 112, 1615-1632.
15. M. Krishnamoorthy, S. Hakobyan, M. Ramstedt, J. E. Gautrot, Chem. Rev. **2014**, 114, 10976-11026.
16. Q. Wei, T. Becherer, S. Angioletti-Uberti, J. Dziubella, Ch. Wischke, A. T. Neffe, A. Lendlein, M. Ballauff, R. Haag, Angew. CHem. Int. Ed. **2014**, 53, 8004-8031.
17. A. Dworak, I. Panchev, B. Trzebicka, W. Walach, Macromol. Symp. **2000**, 153, 233-242.
18. W. Wałach, B. Trzebicka, J. Justyńska, A. Dworak, Polymer **2004,** 45, 1755-1762.
19. A. Mendrek, S. Mendrek, B. Trzebicka, D. Kuckling, W. Wałach, H.-J. Adler, Macromol. Chem. Phys. **2005**, 206, 2018-2026.
20. A. Thomas, S. S. Muller, H. Frey, Biomacromolecules **2014**, 15, 1935-1954.
21. A. Dworak, B. Trzebicka, A. Utrata, W. Wałach, Polym. Bull. **2003**, 50, 47-54.
22. M. Weinhart, T. Becherer, N. Schnurbusch, K. Schwibbert, H.-J. Kunte, R. Haag, Adv. Eng. Mater. **2011**, 13, B501-B510.
23. M. Weinhart, I. Grunwald, M. Wyszogrodzka, L. Gaetjen, A. Hartwig, R. Haag, Chem. Asian J. **2010**, 5, 1992-2000.
24. Ch. Xue, B.-Ch. Choi, S. Choi, P.V. Braun, D.E. Leckband, Adv. Funct. Mater. **2012**, 22, 2394-2401.
25. X. Wang, R. Berger, J.I. Ramos, T. Wang, K. Koynov, G. Liu, H.-J. Butt, S. Wu, RSC Adv. **2014**, 4, 45059-450564.
26. S. J. Sofia, V. Premnath, E.W. Merrill, Macromolecules **1998**, 31, 5059-5070.
27. Y.-J. Kim, Y.T. Matsunaga, J. Mater. Chem. B **2017**, 5, 4307-4321.
28. D. Roy, W. L. A. Brooks, B. S. Sumerlin, Chem. Soc. Rev. **2013**, 42, 7214-7243.
29. A. Dworak, B. Trzebicka, A. Utrata, W. Wałach, Polymer Bulletin **2003**, 50, 47-54.
30. Doycheva, M., E. Petrova, R. Stamenova, C. Tsvetanov, G. Riess, Macromol. Mater. Eng. **2004**, 289, 676-680.
31. K. Nagase, J. Kobayashi, T. Okano, J. R. Soc. Interface **2009**, 6(3), S293309.
32. Z. Hu, T. Cai, Ch. Chi, Soft Matter **2010**, 6, 2115-2123.

33. Y. Tsujii, K. Ohno, S. Yamamoto, A. Goto, T. Fukuda, w Surface initiated polymerization I, Advances in polymer science; J. Jordan, wyd. Springer: Berlin, Heidelberg, **2006**; Vol. 197; p 1-45.
34. R. Hoogenboom, Angew. Chem. Int. Ed. **2009**, 48, 7978-7994.
35. F. C. Gaertner, R. Luxenhofer, B. Blechert, R. Jordan, M. Essler, J. Controlled Release 2007, 119, 291-300.
36. B. Guillerm, S. Monge, V. Lapinte, J. J. Robin, Macromol. Rapid Comm. **2012**, 33, 1600-1612.
37. R. Hoogenboom, H. Schlaad, Polymers **2011**, 3, 467-488.
38. G. Morgese, E. M. Benetti, Eur. Polym. J. **2017**, 88, 470485.
39. M. Meyer, M. Antonietti, H. Schlaad, Soft Matter **2007**, 3, 430-431.
40. P.T. Guner, A. Miko, F.F. Schweinberger, A.L. Demirel, Polym. Chem. **2012**, 3, 322-324.
41. A. L. Demirel, M. Meyer, H. Schlaad, Angew. Chem. Int. Ed. **2007**, 46, 8622-8624.
42. C. Diehl, I. Dambrowsky, R. Hoogenboom, H. Schlaad, Macromol. Rapid Comm. **2011**, 32, 1753-1758.

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