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Modifications of the polymer surface aimed at improving cell adhesion and interaction

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Abstract

The most commonly used biomaterials are polymers – natural (collagen, laminin, chitosan) or synthetic (polylactide, polyethylene oxide, polyglutamate, etc.), which have certain (appropriate) mechanical properties, but most importantly, they are biodegradable. Chemical polymers have recently been preferred and displaced natural ones such as donor skin, collagen, bone implants, etc., as they are cheaper, easier to modify, and largely avoid immunological reactions. The next stage in the development of biomaterials is related to the emergence of bio-hybrid technologies, with the demand for materials that have a positive response to tissues. These are bioactive biomaterials. They are looking for contact with tissues, looking for ways to optimize these interactions. Exploring cell-surface interaction is important for the creation of both bioinnergic and bioactive (hybrid) materials vital to medicine. Despite the efforts made so far, the mechanism of this impact has not yet been fully elucidated. Functioning of polymer surfaces is an approach recently used systematically to modulate their interaction with living cells. It allows to take a deep look into the mechanisms of biocompatibility and to understand the role of the surface properties of polymeric biomaterials for their successful interaction with the body. The resulting new materials would be of great importance for use in medicine and biomedical engineering.

Introduction

The functionalization of polymer surfaces is the basis for the production of new biomaterials which will play an important role in improving biocompatibility.

A number of methods are known and new methods of functionalization have recently been introduced to produce good antibacterial and anti-cancer activity. Basic methods of surface modification can be divided into two main categories: physicochemical and biological. Examples of physicochemical methods are acid treatment, oxidation, grafting polymerization, flame treatment, crown discharge or cold plasma, photolithography, and others. Microarrays on the biomaterial surface create structures of different sizes and shapes that control the spreading of the cell orientation [1]. Hydrophilization is a basic approach to improving cellular interaction as cells prefer the hydrophilic surface. The reason for this is not entirely clear, but probably lies in the conformation of the adsorbed adhesive proteins [2]. Plasma treatment offers one possibility of changing the surface tension and creating highly hydrophilic or hydrophobic surfaces. Carlsson and Johansson (1993) deposited perfluoropropane and ethylene oxide films on PDMS, using a flame discharge in tetrafluoroethylene to give high fluorinated surfaces. Currently, there are many sttempts to optimize the surface biocompatible properties of different polymeric materials by plasma treatment, often combined with classical organochemical reactions. Typical of this type of modification is that in all cases the surface

energy changes and thus influences the interfacial interactions. It has been found, however, that some polymer surfaces have similar free surface energy and different chemical nature [3]. Obviously, surface energy is not the only factor on which the bioconductive properties of the polymeric material depend. Many chemical groups such as hydroxyl, carbonyl, carboxyl, amine, are noted as important factors for modulating the fate of attached cells [4]. For example, the ability of macrophages to form giant, multinuclear cells (granuloma reaction) on the surface of some hydrogels correlates with the presence of certain chemical groups. The likelihood of fusion of macrophages decreases in the following order of chemical groups [5]: ((CH3) 2N-> -OH> -CO-NH-> -SO3H> -COOH> -COONa). A similar hierarchy was also observed in CHO cells incubated on functionalized surfaces where attachment and growth decreased in the following order: -CH2NH2 -CH2OH> -CONH2> -COOH.

Biological methods

Grafting of the cell adhesion peptide Gly-Arg-Gly-Asp (GRGD)

The modification of synthetic polymers having appropriate mechanical properties and processability with biopotential interfering particles similar to those of the ECM (extra cellular matrix) allows to combine the advantages of the synthetic material with many of the advantages of natural materials as well as to stimulate cell-adhesion interactions with cell surface receptors resembling interactions with specific ligands of the EMC [6]. In order to provide a natural emblem-like substrate, besides chemical functional groups, matrix proteins, such as collagen, fibronectin, and the like can be mobilized on the surface of the synthetic polymeric material. This is the essence of the biomimetic approach aimed at resembling some specific features in the structure or functions of the natural extracellular microenvironment [7]. This group of methods includes simple protein adsorption, enzyme immobilization, cell pre-sowing, and others. Other smaller biologically active molecules may also be used to modify the surface, e.g., peptides containing amino acid sequences and integrin receptors of the cell [8]. RGD (Gly-Arg-Asp), found in many cell adhesion proteins and integrin receptors of various types of cells [8], has been most extensively studied. Such peptides are immobilized, for example, on the surface of polytetrafluoroethylene [8], polyacrylamide [9], polyurethane [10], polycarbonate [11], and other substrates. Lin et al. found that improving cell growth depends on grafting density [10]. Also, other biologically active molecules such as polysaccharides, oligosaccharides or glucolipids are used to improve cell adhesion, in addition to adhesive peptides. In some cells, adhesion is increased by the adsorption of homopolymers of certain amino acids such as polylysine and polyornin. Immobilization of polysaccharides on polymeric surfaces affects both cell attachment and over-the-surface functions, as for example in the immobilization of some polysaccharides on polyacrylamide disks. Elbert and Hubbell (1996) cover polyethylene with monoclonal antibodies against cell-membrane antigens and ECM proteins. In this way, they improve the adhesion and proliferation of human endothelial cells. To exemplify the ETC which determines the specific interaction with hepatocytes, Bartolo et al., 2007 modify the surface of a polyetherimidosulfonic membrane by plasma deposition of acrylic acid with subsequent covalent immobilization of RGD peptides through a hydrophilic spacer (linear diamino PEG). The latter binds covalently with one amino group to -COOH on the surface and with the other one, forming a peptide bond with -COOH groups of the RGD peptide [12].

Chemical methods

Processing in plasma

Plasma treatment has emerged in recent decades as a promising method for surface modification of various biomedical engineering materials [13]. The study of cell interaction has shown that collagen binding provides the adequate environment for attachment, growth and migration [14]. Based on this, Lee and collaborators [15] conclude that the polysiloxane membrane, modified by plasma-induced graft polymerization of acrylic acid and subsequent collagen grafting, has enormous potential.

A widely used approach to the development of biomimetic polymer surfaces is the immobilization of bioactive proteins. Collagens are the predominant proteins of the extracellular matrix, and appropriate immobilization on polymeric surfaces while preserving their biological activity could create favorable conditions for the cultivation of different types of cells. Type I collagen is predominant in bone, skin, tendons and sclera. It has been used as an adhesive protein in current research. It is extremely important to immobilize collagen to maintain its biological activity after binding to the polymer surface.

Coating of PEG (poly ethylene glycol) surfaces

Polyethylene glycol (PEG) is a non-ionic, water-soluble oligomer used extensively to stabilize colloids in the food, lacquer, pharmaceutical and cosmetic industries. Recently, interesting potential uses of PEG in biotechnology and medicine have been described [16]. Surfaces coated with PEG have been found to exhibit no antigenic activity. This is used to mask medications that could otherwise cause allergic reactions. Other important areas of application of PEG are as carriers of cytostatic agents in the prophylactic therapy and the use of PEG / dextran mixtures in affinity separation of proteins. Incorporating PEG in proteins preserves their biological activity even when the protein films are stored in air. In this case, PEG retains the moisture and thus preserves the natural environment of the protein. The molecular origin of the specific PEG properties is too complex. It is assumed [16] that the structural similarity of the oxyethylene-CH_2CH_2O- and the water molecule (dipole moment, medium length non-linear link and angle of the -C-O-C- bond) hydrogen bond with an O atom, favor their mixing. Furthermore, the -CH_2- groups are flanked by water molecules bound by two to each oxyethylene unit. Therefore, when PEG is an aquatic environment, any foreign body (proteins, platelets, cells, etc.) is actually in contact with the molecules of water surrounding the oxyethylene chain. Because of the unique equilibrium in water structure and PEG molecules, no adsorption of the water / PEG interfacial boundary caused by dehydration (such as hydrophobic materials) is expected.

Surfaces coated with oxyethylene groups are also promising in terms of improving biocompatibility. Particularly interesting are its repellent properties with respect to coagulation cascade proteins (coagulation) and complement. Recently, it has been found that in a certain PEG binding architecture to the surfaces it is possible to modulate the interaction of the cells. Under certain conditions, PEG is repellent for cellular proteins, but in others it can dramatically enhance cell adhesion. Such dependence is observed in relation to the length of the PEG chain as well as to its density on the substrate. PEG-coated surfaces activate the complement system [4] and reduce adsorption stains [4]. Therefore, PEG surface transplantation modifies the interaction with mammalian cells [18].

Features of PEG coatings

The properties of PEG coatings: chemical stability, thickness, structure, and composition, depend largely on how they are prepared. Coating of a PEG-rigid surface with a thick PEG is difficult because, at temperatures below the temperature degradation in an aqueous medium (the darkening point), PEG molecules naturally repel. The most widely used are three experimental methods for preparing PEG coatings:

- deposition of hydrogel or mechanically stable PEG coatings by direct photopolymerization of an appropriately functionalized PEG (most commonly, monoacrylate or methacrylate) [18] on the polymer surface;

- chemical grafting of functionalized PEG to a preactivated polymer surface – amino-aldehyde and amino-epoxy attachment are most commonly used [18].

- quasi-reversible adsorption of a functionalized PEG to a suitably activated surface, e.g., deposition of PEG-epoxide on a pre-amine surface or pre-prepared adduct PEG-epoxide and polyethyleneimine [19].

In addition, biomaterials can be modified with biological molecules. Biomaterials include simple protein reabsorption, enzyme immobilization, cell pre-sowing, etc. Other less biologically active molecules, the so-called adhesive proteins such as fibronectin, can be used to modify surfaces. They are distinguished from integrin receptors and mediate cell adhesion. Cells are well attached to surfaces containing the adsorbed oligopeptide RGD sequence, the active matrix with which the adhesive proteins interact. Such peptides have been immobilized to polytetrafluoroethylene, polyacrylamide polyurethane [20], polycarbonate tartrate [21], polyethylene glycol [21] and other substrates. The addition of RGD induces cell adhesion and helps spread and form focal adhesion contacts on non-adherent polymers [8]. On the other hand, the different cells contain a different set of adhesive receptors that recognize certain EMC molecules. Therefore, the immobilization of suitable cell-binding proteins can lead to the creation of cell-selective surfaces. In summary, surface functionalization is an approach that is systematically employed by various researchers to improve their biocompatibility and deeper penetration into the mechanisms of interaction of a living cell with "foreign" organisms' surfaces.

Polydimethylsiloxane (PDMS)

In many studies, the stability, toxicity, hydrophobicity, tissue response, and oxygen permeability of siloxane elastomers are discussed. The lack of bioavailability, softness, stability, and transparency of these elastomers as well as their implantation for a long time, cause serious problems because the cells do not interact well with hydrophobic materials as they are. Such problems naturally give rise to the need for surface modification for their hydrophilization. The literature describes a number of methods for modifying the surface of siloxane rubbers, leading to an improvement in their biocontact properties, and the possibilities in this regard are far from exhausted. It is considered that surface modification will contribute to solving the problems of interfacial contact and will lead to the expansion of biomedical applications of this material [21].

The modification methods described in the literature generally include wet chemistry, plasma treatment, ion bombardment, laser irradiation, etc., leading to modification of the chemical composition of the surface, surface energy, hardness, crosslinking, roughness, hydrophilic / hydrophobic balance etc., which ultimately lead to a change in the biocontact properties of this material. Due to its relatively good biocompatibility, superior flexibility and resistance in biological media, siloxane rubber is the preferred material Abbott continuously uses in catheters. Their serious disadvantage, however, is their tendency to biopollination and thrombus formation, which could cause their occlusion. A number of studies [22] show that the implantation of argon ions at the surface significantly reduces the friction and bio-contamination of siloxane rubber and could be used to prevent the aforementioned deficiencies of the siloxane catheters. In recent decades, plasma treatment has emerged as a promising method for surface modification of various biomedical engineering materials [13]. The study of cell interaction shows that collagen binding provides the adequate environment for attachment, growth and migration [14]. On the basis of this, Lee et al. (1996) concluded that the cipolysiloxane membrane modified by plasma-induced graft polymerization of acrylic acid and subsequent grafting of collagen has a tremendous potential. A widespread approach to the development of biomimetic polymer surfaces is the immobilization of bioactive proteins. The collagen extracellular matrix proteins and the appropriate imiquillization on polymeric surfaces while preserving biological incitement could create favorable conditions for the cultivation of different types of cells. Type I collagen is predominant in bone, skin, tendons, and sclera. It has been used as an adhesive protein in current research. It is extremely important to harvest collagen by preserving its biological activity after binding to the polymer surface. Therefore, binding of the protein to the polymer is often done through a flexible chain. PEGs are usually used as such a chain, as they have an active free end capable of interacting with an active end of the protein. Preferably, collagen binding is made to the less accessible carboxyl groups in order to preserve the conformational freedom of its molecules, which is critical to the possibility of reorganization by the cells. Due to the simplicity of production and their low cost, the interest to PDMS microfluidic articles is constantly increasing, but at the same time, the need for strategies for modulation of surface properties increases.

Grafting of acrylic acid, acrylamide, dimethyl acrylamide, 2-hydroxyethyl methacrylate and polyethylene glycol monomethoxy acrylate reduces the water wetting angle to below 450^0, resulting in improved electroosmotic motility in the micro channels.

The use of a polymeric material in contact with tissues, blood, and other biological fluids requires the solving of serious problems associated with its compatibility. The regulation of the interaction between material and living matter (tissues, blood, cells, etc.) is the most serious problem of modern polymer chemistry. This interaction is most often accompanied by side effects leading to inflammatory processes and necroses, blood coagulation, thrombus formation, or implant rejection reaction. The biocompatibility, as well as the specificity of the bio-interaction, adhesion and wetting, wear-resistance and appearance, could be optimized by modifying the polymer surface. Surface functionalization is an approach that is systematically used in the work of many researchers studying the interaction of cells with biomaterials. This is the main approach in our research as it allows us to take a deeper insight into the mechanisms of cell attachment to polymer surfaces and to understand the determining role of the surface properties of biomaterials upon their interaction with cells [15]. Initial interaction of cells with material surfaces can be reduced to the cell

adhesion process. An important property of cell adhesion is its selectivity. Cells, as well as all intracellular structures, must be attached and oriented in space, as a point of reference is needed to move them. The ability to attach is a basic property of all living cells that determines their vital activity. Under conditions *in vitro*, cells can be attached to different surfaces - a process that is relevant to their interactions with biomaterials [15]. The mechanism of interaction of cells with artificial surfaces has not been fully studied, but factors such as hydrophilic / hydrophobic balance, surface roughness, type and amount of some surface functional groups play an essential role. Generally, hydrophilic surfaces are preferred in the creation of bio-hybrid bio-materials, and hydrophilisation is the basic and commonly accepted approach to improving cellular interaction. The reason for this effect of hydrophilicity is most likely contained in the conformation of adsorbed adhesion proteins favoring cell adhesion. There are numerous possibilities for superficially functionalizing polymers, generally causing a change in their surface free energy and the generation of uncharged, positively or negatively charged, as well as containing different functional groups of surfaces [15]. For example, immobilization of hydrophilic chemical groups on the surface of the polymer could improve its interaction with cells. There are a variety of options for this, but it is very important that the process is carried out in such a way as to avoid disturbing the biological activity of the surface. In our studies, a difference in cell interactions in the different PEG coatings, such as PEG 1500, PEG 6000 and PEG 12500, was observed, which can be explained by the specific organization of PEG chains, their length, structure, and the different hydrophilic / hydrophobic balance of these substrates. It should be noted, however, that the cells interact directly with the substrate. In order to maintain their physiological attachment, they need adhesion proteins or other attachment factors (polysaccharides) to be adsorbed from the environment. Such a factor is, for example, fibronectin [23]. Here, the good biological properties of PEG 6000 (on which the highest FH adsorption was measured), while the short-cut PEG 1500 showed low FH adsorption, corresponding to a poor cell interaction as determined by the fibroblast assays. For PEG 12500 the effect of FN pre-adsorption was strongest, but it should be noted that the initial cell adhesion (to the pure PEG 12500) was poor. In general, PEG 12500 appears to have very good biological properties but only if it is pre-absorbed with fibronectin. The lowest FN adsorption was also found on this polymer. There is no direct relationship between the amount of preadsorbed fibronectin on PEG surfaces and their biological properties, which corresponds to the basic notion that the amount and conformation of the adsorbed protein molecules is important for cell interaction. In general, it should be taken in consideration that lower protein adsorption does not necessarily mean lower cell adhesion and spread [23]. Once the FN was isolated and identified as a serum component responsible for cell adhesion *in vitro* [24], the improved biological activity of the materials began to be explained by the different way of adsorption of the FN. It is well known that the cells on hydrophobic surfaces cannot reorganize the adsorbed FN [25], which is probably related to their stronger attachment to the substrate. Interestingly, in our PEG surface study, we observed poor protein adsorption but surprisingly good reorganization, especially on PEG12500 surfaces, which is an example of how weak variations in the polymeric structure can have a significant effect on adhesion molecule behavior. Interestingly, despite the extremely poor protein adsorption, PEG coatings allow interaction with cells which depend on both the PEG polymer structure (long chain, short-chain, branched) and the density of the coating. Our studies have shown that longer PEG chains offer better conditions for cell attachment, and it is the FN that determines this interaction because in its absence adhesion is weak. The probable explanation for this effect is that the FN adsorbs not directly to PEG that is highly protein-replicating [16] butto the remaining free binding sites on the substrate. In this situation, PEG chains exert a significant stabilizing effect on the conformation of adsorbed proteins due to its hydrophilic nature. Searching for approaches to hydrophilize the surface of biomaterials is a step towards improving their bioavailability. One of these approaches is Ar + plasma treatment [25], and the results from the synthetic rubber studies are a typical example of this. As explained in the introduction, this material has a great potential for biomedical use but is hydrophobic and shows poor biocompatibility. Plasma treatment of pure PDMS definitely resulted in the hydrophilization of its surface to about WCA 60^0, changing it from hydrophobic to moderately hydrophilic. The mechanism of this process is related to surface functionalization and change of free energy [25], which is believed to lead to an improvement in biocompatibility. Interestingly, cellular interaction with plasma-treated PDMS occurs even without FH, and this can be explained by the easier overcoming of the electrostatic barrier that favors the attachment of cells [25]. The chemical bonding of collagen I to PDMS is another way to improve cellular biocompatibility. This multilevel process involves processing in Ar + plasma, grafting of acrylic acid (AA), and PEG as a flexible linkage for binding of the PDMS to the collagen molecules through [25]. The fundamental change in the composition of the chemical surface (its mineralization) due to processing in Ar + plasma occurs in the first minute after processing of the MPD at 1200 W, as was found in previous studies [25]. Therefore, samples for further grafting with AA were processed under these conditions (1200 W / l min). A flexible PEG link with different chain lengths was grafted onto flexible AA samples, followed by collagen binding 1. Three diNH2-PEGs with a relatively long polymeric chain (2000, 6000, and 20000 Da) were used, and we expected that the relatively long flexible PEG spacer would provide greater freedom for the collagen molecules attached and better interaction with fibroblasts [25].

In our research we used different methods to characterize the resulting surfaces. One of these is X-ray photoelectron spectroscopy or XPS analysis. Here we will discuss it briefly in order to better explain the interaction of the cells with the various modified PDMC surfaces. The X-ray shows a comparison of the chemical composition of unmodified and Ar + plasma-treated PDMS. The treated CSF shows a change in the surface chemical composition compared to the untreated one: the oxygen content increases to 49.4%, along with a significant reduction in carbon content (up to 18.6%), both Si / C and Si / O ratios changing significantly after treatment in argon plasma. The associated AA changes further the surface chemical composition of plasma treated PDMS: silicon content decreased to 28%, and both Si / C and Si / O relationships changed again. A new component was observed, indicating the presence of -COO groups on the surface [25]. Namely, these carboxyl groups of AA-attached to PDMS were used to attach diNH2-PEG to serve as a flexible bond between the surface and the collagen. The covalent attachment of diNH2-PEG to the surface was confirmed by the detection of a significant amount of nitrogen on the diNH2-PEG-coated surfaces [25]. In a more detailed review of the XPS analysis data, the Nls spectrum has shown amine and amide groups on the surface. These groups arise as a result of the interaction between PEG-spider's NH2-groups and -COOH of AA-

grafted surface and demonstrate the formation of a peptide bond. The nitrogen content is the highest (4.4%) on PEG 6000. For the same surface, it is also noted that the density of the NH2 groups is the highest. There is evidence that the presence of NH_2 groups on the surface enhances the interaction of cells with polymeric surfaces [3], therefore, we can conclude that the best interaction with fibroblasts is where a flexible spacer PEG 6000 is used. A demonstration of the successful attachment of collagen comes from the presence of low sulfur, which was shown by the XPS analysis. This result can be explained by the presence of sulfur-containing amino acids in collagen such as methionine cysteine [26]. XPS data was confirmed by contact angle measurements. The water contact angle (WCA^0) of the highly hydrophobic PDMS (101.9^0) significantly decreases at each stage of the modification, and the surface becomes more or less hydrophilic depending on the type of treatment [25].

In addition, an Atomic Power Microscopy (ACM) study was performed to obtain more detailed information on the surface morphology and roughness of the treated PMDM. Thus, the surface of the unmodified PDMS has been found to be relatively smooth and shows a fine-grained surface topography similar to that observed by other authors. Surface topography is almost retained after treatment with Ar + plasma interaction with polymer surfaces [25]. Comparison of the ACM images clearly shows that surface abnormalities increase sharply after AA grafting [25]. Surface irregularity also increases with the addition of PEG, proven by XPS analysis and measurements of the water contact angle. The length of the PEG chain also affects the surface irregularity and the collagen-bonded surfaces. Thus, the longer the PEG chain, the lower the surface roughness and the less interaction of cells with this surface, as cells are known to predict rough surfaces. Here, however, we must take into consideration the effect of collagen as a major component of basal membranes, which is specific to cells. After examining the chemical and physico-chemical analysis data of the treated PDMS, we can explain much more about the different cell behavior on the modified surfaces. For example, the different number of fibroblasts adhered to the three PEG surfaces can be explained by the simultaneous influence of hydrophilicity and surface roughness, both depending on the length of the PEG chain. Cellular behavior, however, is different after attaching collagen I to these surfaces. Obviously, the adhesion and distribution of fibroblasts are positively influenced by collagen modification, yet cell adhesion also depends on the length of the PEG chain used as a flexible spacer. The length of the PEG chains is poorly influenced by the surface hydrophilicity of the collagen-modified surface. Moderate hydrophilicity (θH_2O = 40.0-47.5 °), which is a prerequisite for good interaction with living cells [25], may also cause improved cellular interaction of the examined collagen-coated PDMC surfaces. In addition, the flexible spacer chain may provide a suitable conformation of the collagen molecule and thus expose its cell binding sequences (GFOGER, RGD) more readily, and this also results in better interaction with the cells. It is interesting to note that cells normally interact with the native collector by their α1β1 and α2β1 integrin receptors, recognizing the GFOGER sequence, i. e. by one RGD independent mechanism, and in order to expose the RGD sequence of the collector molecule, its partial denaturation is necessary [27]. But then collagen also begins to interact with α5β1 and αvβ3 integrins that are specific for other protein proteins (fibronectin and vitronectin) [28], and although cellular adhesion exists, it may also trigger other signaling pathways.

Conclusion

To summarize, there are different ways to modify a polymer surface. Basically, there are biological and physicochemical methods. Our experience and studies have shown that Ar + plasma treatment (physicochemical treatment) opens a new opportunity for biofunctionalization of PDMS, a multi-step procedure ending with the immobilization of collagen by using a flexible PEG connection of an average length of 6000 Da, which is preferable due to the optimal initial cell adhesion and proliferation. However, there was no direct correlation between hydrophilicity and initial cellular interaction, probably due to the simultaneous influence of other factors such as superficial chemical structure and topography [25]. Because of the simplicity of production and their low cost, the interest in synthetic materials like PDMS and PEG keeps growing constantly, but at the same time there is an increasing need for new methods of modifying the properties of polymer scaffolds. Future developments in optimizing biomaterials will be directed to the creation of polymers and nanostructure materials with various metal ions which lead to its transformation into a promising antibacterial material. Metal ions such as copper, silver, titanium and others improve antibacterial properties on the surface but at the same time remain biocompatible. In recent years, polymeric surfaces have been created by functionalization in order to respond to the challenge and to develop synergistic biomedical engineering and regenerative medicine.

References

1. Flemming RG, Murphy CJ, Abrams GA, Goodman SL, Nealey PF. Effects of synthetic micro- and nano-structured surfaces on cell behavior. Biomaterials. 1999; 20: 573-588.
2. Grinell F, Feld M. Adsorbtion characteristics of plasma fibronectin in relationship to biological activity. J Biomed Mater Res. 1981; 15: 363-381.
3. Lydon MJ, Minett TW, Tighe BJ. Cellular interactions with synthetic polymer surfaces in culture. Biomaterials. 1985; 6: 396-402.
4. Gölander G, Lassen B, Nilsson K, Nilsson U. RF- plasma-modified polystyrene surfaces for studing complement activation. J Biomater Sci Polym Edn. 1992; 4: 25-30.
5. Smetana KJ. Cell biology of hydrogels. Biomaterials. 1993; 14: 1046–1450.
6. West JL. Biofuntional Polymers, Encyclopedia of Biomaterials and Biomedical Engineering. 2007; 89-95.
7. Chen R, Hunt A. Biomimetic materials processing for tissue-engineering processes. J Mater Chem. 2007; 17: 3974–3979.
8. Massia SP, Hubbell JA. An RGD spacing of 440 nm is sufficient for integrin alpha V beta 3-mediated fibroblast spreading and 140 nm for focal contact and stress fiber formation. JCB. 1991; 114: 1089-1100.
9. Brandley B, Schnaar R. Covalent attachment of an Arg-Gly-Asp sequencepeptide to derivatizable polyacrylamide surfaces: support of fibroblast adhesion and longterm growth. Anal Biochem. 1988; 172: 270-278.
10. Lin YS, Wang SS, Chung TW. Growth of endothelial cells on differentconcentrations of Gly-Arg-Gly-Asp photochemically grafted in polyethylene glycol modified polyurethane. Artif. Organs. 2001; 25: 617–621.
11. Drumheller PD, Elbert DL, Hubbell JA. Multifunctional Poly(ethylene glycol)Semi-Interpenetrating Polymer Networks

as Highly Selective Adhesive Substrates for Bioadhesive Peptide Grafting. Biotechnology and Bioengineering. 1994; 43: 772-780.

12. Bartolo LD, Morelli S, Piscioneri A. Novel membranes and surfacemodification able to activate specific cellular responses. Biomolecular Engineering. 2007; 24: 23–26.

13. Szicher M, Sioshansi P, Frish E. Biomaterials for the: Polyurethanes, Silicones and Ion Beam Modification Techniques´ (Part 2). Patriots Park Bedford. 1990.

14. Chu PK, Chen JY, Wang LP, Huang NK. Plasma-surface modification of biomaterials. Mater Sci Eng. 2002; 36: 143–206.

15. Lee SD, Hsiue GH, Kao CY. Preparation and characterization of a homobifunctional silicone rubber membrane grafted with acrylic acid via plasma induced grafted copolymerization. J Polym Sci A Polym Chem. 1996; 34: 141–148.

16. Harris JM. Poly (Ethylene) Glicol Chemistry-Biotechnical and biomedicalapplications Plenum Press: NY. 1992.

17. Yamamoto Y, Sefton MV. Surface grafting of poly(ethylene glycol) onto poly(acrylamide-co-vinyl amine) cross-linked films under mild conditions. J Biomat Sci Polim Edn. 1998; 9; 427-437.

18. Kish E, Gölander G, Eriksson JC. Surface grafting of polyethyleneoxide optimized by means of ESCA, Progress in Colloid and Polymer Science. 1987; 74: 113–119.

19. Lee J, Kopeckova H, Kopecek P, Andrad JD. Surface properties of copolymers of alkyl methacrylates with methoxy (polyethylene oxide) methacrylates and their application as protein-resistant coatings. Biomaterials. 1990; 11: 455-64.

20. Lin H, Sun W, Mosher F, Garcia-Echeverria C, Schaufelberger K, et al. Synthesis, surface, and cell-adhesion properties of polyurethanes containing covalently grafted RGD-peptides. J Biomed Mater Res. 1994; 28: 329-342.

21. Breuers W, Klee D, Hocker H. Immobilization of a fibronectin fragment at thesurface of a polyetherurethane film. J Mat Sci: Materials in Medicine. 1991; 2: 106-109.

22. Sioshansi P, Tobin EJ. Surface treatment of biomaterials by ion beam processes. Surf Coat Technol. 1996; 83: 175-182.

23. Grinnell F, Ho CH, Tamariz E, Lee DJ, Skuta G. Dendritic fibroblasts inthree dimensional collagen matrices. Mol Biol Cell. 2003; 14: 384-395.

24. Klebe RJ. Isolation of a collagen-dependent cell attach- ment factor. Nature. 1974; 250: 248-251.

25. Keranov I, Vladkova T, Minchev M, Kostadinova A, Altankov G. Preparation, characterization, and cellular interactions of collagen - immobilized PDMS surfaces. J Appl Polym Sci. 2009; 110: 321-330.

26. Ayad S, Boot-Hanford RP, Humphries MJ, Kadler KE, Shuttleworth CA. The Extracellular Matrix Facts Book, Academic Press, London, San Diego. 1994; 8671.

27. Yamamoto M, Yamato M, Aoyagi M, Yamamoto K. Identification of integrins involved in cell adhesion to native and denatured type I collagen and the phenotypic transition of rabbit arterial smooth musckle cells. Exp Cell Res. 1995; 219: 249–256.

28. Davis GE. Affinity of integrins for denatured extracellular matrix: alpha-v beta 3 binds to denatured collagen type 1 through RGD sites. Biochem. Biophys. Res. Commun.1992; 182: 1025-1031.

29. Carlsson C, Johansson K. Surface modification of plastics by plasma treatmentand plasma polymerization and its effect on adhesion. Surf Inter Anal. 1993; 20: 441.

30. Elbert DL, Hubbell JA. Surface Treatments of Polymers for Biocompatibility. Annu Rev Mater Sci. 1996; 26: 365-394.

31. Hlady V, Van Wagenen RA, Andrade JD. “Surface and Interfacial Aspects of Biomedical polymers”. Editors. Andrade JD. Plenum Press, New York. 1985; 2: 81.

32. Lin HB, Zhao ZC, Garcia-Echeverria C, Rich DH, Cooper SL. Synthesis of a novel polyurethane co-polymer containing covalently attached RGD peptide. J Biomater Sci Polymer Ed. 1992; 3: 217-227.

33. Taipale J, Keski-Oja J. Growth factors in the extracellular matrix. FASEB J. 1997; 11: 51-59.