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Research review paper

Nano-structured and functionalized surfaces for cytocompatibility improvement and bactericidal action

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ABSTRACT

The field of material surface modification with the aim of biomaterial construction involves several approaches of treatments that allow the preparation of materials, which positively influence adhesion of cells and their proliferation and thus aid and improve tissue formation. Modified materials have a surface composition and morphology intended to interact with biological systems and cellular functions.

Not only surface chemistry has an effect on material biological response, surface structures of different morphology can be constructed to guide a desirable biological outcome. Nano-patterned material surfaces have been tested with the aim of how surface geometry and physical properties on a micro- and nano-scale can affect cellular response and influence cell adhesion and proliferation.

Biological functionality of solid state substrates was significantly improved by the irradiation of material with plasma discharge or laser treatment. Commonly used "artificial" polymers (e.g. polyethylene (PE), polystyrene (PS), polytetrafluoroethylene (PTFE), polyethylene terephthalate (PET), polyethylene naphthalate (PEN)) and biopolymers (e.g. Poly-L-Lactic acid (PLLA), polymethylpentene (PMP)) were treated with aim of biocompatibility improvement. The treatment of polymer/biopolymer substrates leads to formation of ripple or wrinkle-like structures, supported also with heat treatment or other subsequent surface processing. Several types of chemically different substances (e.g. metal or carbon nano-particles, proteins) were grafted onto material surfaces or built into material structures by different processes.

Surface physico-chemical properties (e.g. chemistry, charge, morphology, wettability, electrical conductivity, optical and mechanical properties) of treated surfaces were determined. The enhancement of adhesion and proliferation of cells on modified substrates was investigated in vitro. Bactericidal action of noble metal nano-particles (e.g. Au, Ag) on polymers was characterized. The influence of metal nano-particle grafting by using metal nano-particle suspension prepared by "green" methods was determined. Micro- and nano-patterned surfaces can be constructed as tissue scaffolds with specific functions regarding cell adhesion and proliferation or potential biosensor applications.

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1. Introduction

Polymeric materials can be used for construction of replacements of irreversibly damaged tissues and organs. As the best replacements (or the "golden standard" for all replacements), the autologous tissue is still considered. However, obtaining this tissue is associated with several drawbacks, such as limited availability, additional surgery for the patient and donor site morbidity. Allogenous and xenogenous transplants are burdened with a risk of immune rejection and disease transmission, and if the donor tissue is preserved by chemical agents crosslinking (e.g. glutaraldehyde), also with potential toxic damage of the recipient's tissues (for a review, see Filová et al., 2009; Chlupáč et al., 2009). Thus, a new advanced interdisciplinary field called "tissue engineering" is quickly developing, and according its classical definition, it "applies the principles of engineering and the life sciences towards the development of biological substitutes that restore, maintain, or improve tissue function" (Langer and Vacanti, 1993).

The tissue substitutes created by methods of tissue engineering contain a cell component and a material component, which serve as a carrier for the cells since they are generally anchorage-dependent. The material component can comprise either synthetic molecules (e.g., various synthetic polymers, ceramics) or biological molecules (e.g., polysaccharides or proteins, which are often molecules of the extracellular matrix, such as collagen, fibronectin etc.; see Bačáková and Švorčík, 2008; Bačáková et al., 2004, 2014). For advanced tissue engineering, the biological molecules are used as recombinant. This means that specific human proteins or their functional parts are expressed and synthesized in bacteria, such as *Escherichia coli* (E. coli), in a defined, tunable and reproducible form. This technology also enables to avoid the use of allogeneic and xenogeneic proteins which have the same problems as the allogeneic and xenogeneic transplants of the whole tissue (for a review, see Romano et al., 2011).

Irrespective of the fact if the material designed for body implantation is biological, synthetic, organic or inorganic, it is referred as "biomaterial", because it enters in interaction with biological environments in vivo or in vitro. The biomaterial should operate as an analogue of native extracellular matrix. A relative simple and commonly used way how to regulate the cell behavior by a material is to modulate its physico-chemical properties, e.g. chemistry, polarity, surface energy, wettability, morphology, pH, zeta potential, or rigidity and deformability (Bacakova et al., 2011).

The physico-chemical changes induced on solid state surfaces (e.g. polymers) due to different types of treatment procedures (heating, grafting, laser plasma or ion beam procedure), can be characterized e.g. by microscopic methods (atomic force microscopy (AFM), scanning electron microscopy (SEM), transmission electron microscopy (TEM)), ellipsometry, spectroscopic methods (e.g. Fourier transform infrared spectroscopy (FTIR), Rutherford backscattering spectrometry (RBS), X-ray photoelectron spectroscopy (XPS), UV–vis, Raman spectroscopy), gravimetry, electrical properties, electrokinetic potential and goniometry, allowing the detailed study of changes induced by the treatment. The material surface wettability is one of the most commonly used factors for characterization and tuning of the material. The material wettability is usually generated by the material polarity and surface free energy, and it is directly proportional to these properties. The material surface wettability can be achieved by chemical treatment, e.g. acid, alkali or hydrogen peroxide treatment, which leads to the material oxidation, namely the formation of oxygen-containing chemical functional groups which are polar and thus they produce the material wettability (Wang et al., 2011; Zhang et al., 2011). An alternative approach is physical treatment by irradiation with ion beam (Bačáková et al., 2001a), ultraviolet light (Mikulíková et al., 2005) or laser (Koufaki et al., 2011; Mirzadeh et al., 2011), or by exposure to plasma (Novotná et al., 2013; Parizek et al., 2009). The physical treatment is advantageous especially in synthetic polymers. The common consequences of this treatment is splitting the polymer chains, namely the C – H and C – C bonds, followed by the release of hydrogen, formation of conjugated double bonds in the polymer chains, and particularly by the creation of "oxygen groups" on the material surface. In addition, these treatments often produce a nanostructure of the substrate, which also supports the cell adhesion and growth (for a review, see Bačáková and Švorčík, 2008; Bacakova et al., 2011).

On moderately hydrophilic materials, the cell adhesion-mediating proteins, such as vitronectin, collagen, and laminin, are adsorbed from biological liquids (cell culture medium, blood, interstitial fluid) in a flexible, reorganizable, near-physiological conformation, advantageous for accessibility of specific bioactive spots in above spoken molecules (e.g. RGD) to cell adhesion receptors (of integrin and non-integrin families). Moreover, hydrophobic surfaces promote preferential albumin adsorption, which is poorly adhesive for cells (Bacakova et al., 2011; Bačáková et al., 2004). However, on highly hydrophilic surfaces, the cell adhesion is also low or disabled, because these surfaces prevent the protein adsorption, or the adsorption forces are weak and unstable (Bačáková et al., 2007a; Proks et al., 2012).

Another important material surface property is its roughness and morphology (Miller et al., 2004). In scientific literature, the roughness is most often described by R_a value, described as "the average deviation of the roughness profile from the mean line", that in fact reflects the size of the irregularities, i.e. the height of the prominences and the depth of the depressions (Bačáková et al., 2007b; Vandrovcová et al., 2012). It can be summarized that the macro roughness (size of the irregularities hundreds of μm and more) do not hamper the cell adhesion and spreading, because the cells usually spread over the distances of tens of μm only, and thus they can spread on the side walls of the irregularities on in valleys among them, and do not feel these irregularities. In addition, in case of the bone implants, the macro scale irregularities help to anchor mechanically the implant in the tissue and support its primary stability. The micro scale surface roughness (1 μm to 100 μm) is a more controversial issue. In some studies, it supported the cell's growth and adhesion, while in others it hampered the cell spreading and proliferation, although the lower proliferation activity was often associated with increased cell differentiation (Bacakova et al., 2011; Vagaská et al., 2010; Vandrovcova et al., 2008). Thus, for exact description of the micro roughness, the R_a parameter seems to be insufficient. Also other parameters has to be mentioned, particularly the shape and the distance of the irregularities. If the irregularities are rounded and relatively distant, they may have beneficial or neutral influence on cell spreading and growth, while sharp and densely distributed irregularities may attenuate these properties.

The nanoscale surface roughness (R_a less than 100 nm) is one of the most frequently studied material properties (Vandrovcová and Bačáková, 2001). The reason is that the nanostructured materials usually act as carriers supporting cell adhesion or proliferation. An explanation is that nanoscale irregularities mimic the irregularities in the native extracellular matrix molecules, i.e. their undulations, bending, branching etc., and also irregularities on membrane of cell. In addition, nanoscale surface roughness generates a higher surface wettability due to the larger surface area produced by the irregularities. Addition of nanoparticles (e.g. carbon nanotubes) to an originally highly hydrophobic polymer (a terpolymer of polytetrafluoroethylene, polypropylene and polyvinyldifluoride) and creation of its surface nanoroughness compensated its surface hydrophobicity and significantly increased the number and spreading of cells (Bačáková et al., 2007b; Staňková et al., 2014). The nanoscale surface roughness is considered as advantageous particularly for bone implants preparation and for the prevention of encapsulation of these implants with fibrous tissue (Bacakova et al., 2011; Price et al., 2004; Vagaská et al., 2010; Vandrovcova et al., 2008; Webster et al., 2001).

A certain controversy in the literature is apparent also for the influence of the material's surface electrical charge on the cell behavior.

Some studies indicated that the positively charged surfaces increased the cell adhesion and growth better than the negatively charged surfaces (Kolská et al., 2014). On the other hand, other studies reported a better influence of the negatively charged surfaces on cell colonization (Tarafder et al., 2011). The human osteoblast cells cultured on thermally oxidized TiNb alloys and Ti suggested that the positive charge was associated rather with the cell proliferation, while the negative charge supported more the osteogenic cell differentiation (Jirka et al., 2013). Suchlike results were reported in rat marrow stromal cultures on positively or negatively charged ITO (indium tin oxide) (Qiu et al., 1998). In addition, the increase in negative charge on Ti_6Al_4V alloy after treatment with plasma or heat accelerated the osteogenic differentiation of mouse MC3T3 osteoprogenitor cells in cultures on the material (Rapuano et al., 2012).

The surface charge is related to the pH of the material surface. For example, oxidized materials can contain both acidic and basic hydroxyl (−OH) groups on their surface. In thermally oxidized titanium, acidic groups produced the negative charge, while basic OH groups endowed the surface with positive charge (Feng et al., 2002). The acidic and basic −OH groups tended to act as exchange sites for cations and anions, respectively. Both types of groups supported the deposition of calcium phosphates on the material surface (Park et al., 2007a,b). Both types of groups increased the number of attached osteoblasts, but the basic groups appeared to be more important for these cell functionalities (Feng et al., 2002). At physiological pH, the electrokinetic zeta potential of thermally oxidized TiNb alloy samples was less negative than of the corresponding Ti samples, which was associated with higher proliferation of osteoblast on TiNb samples and higher osteogenic cell differentiation on Ti samples (Jirka et al., 2013).

The electrical conductivity of the material has been unambiguously reported to have a positive influence on the cell adhesion, growth and differentiation. For example, the adhesion and growth of mouse myoblast C2C12 cells and their fusion into myotubes in cultures on hydroxyapatite-calcium titanate composites (HA-$CaTiO_3$), systematically increased with the substrate conductivity due to the addition of $CaTiO_3$ to HA (Thrivikraman et al., 2013). The presence of electro conductive carbon nanotubes in composites of poly(3-hydroxybutyrate) and bioactive glass particles increased cell response in cultures on these materials (Misra et al., 2010). Other materials with carbon nanotubes, namely silk and fibronectin, enhanced the peripheral nerve regeneration in vivo when implanted into rats in the form of nanofibrous nerve conduits (Mottaghitalab et al., 2013). Earlier was reported that NCD (nanocrystalline diamond) layers which were rendered to be electrically conductive by boron doping, supported the cell growth and early osteogenic cell differentiation at lower and medium levels of doping (133 and 1000 ppm B), and the development of focal adhesion plaques and late osteogenic cell differentiation at higher levels of doping (6700 ppm B; Grausová et al., 2011).

Last but not least, a very important material surface property, which has a significant role in the cell fate determining, is rigidity and deformability of the material surface. On very soft surfaces (modulus of elasticity E ~ 1 kPa), the anchorage-dependent cells underwent apoptosis, because they were not able to adhere and spread on these surfaces, even in the case if these surfaces were functionalized with collagen, i.e. a cell adhesion-mediating protein containing ligands for adhesion receptors of cells. Reason was that the material was not able to resist the forces generated by the cell adhesion apparatus (i.e., actin cytoskeleton connected to the adhesion receptors) during cell spreading, and collapsed under the cells (Engler et al., 2004). Slightly stiffer materials, if the rigidity is increased, can induce the cells to be differentiated towards muscle cells (E ~ 8 to 12 kPa) and finally towards osteoblasts (E ~ more than 25 kPa, Engler et al., 2006).

A sophisticated way how to regulate the cell behavior on biomaterials (and how to bring them closer to the native ECM) is their functionalization with ligands for cell adhesion receptors. The most frequently used ligands are synthetic ECM-derived oligopeptides, although some ligands for non-integrin adhesion receptors can be also of saccharide character (Labský et al., 2003). The most known and most frequently used oligopeptides are those containing the RGD sequence, which are recognized by a wide range of cell types. However, some other ligands are specifically or at least preferentially recognized by a single cell type (Bačáková and Švorčík, 2008).

A special issue is the micropatterning or nanopatterning of the material surface. The reason is to create microdomains or nanodomains with different properties (most frequently with domains of different wettability, with periodically arranged depressions and prominences, with domains containing and not containing various biomolecules etc.). The micropatterned surfaces are able to regulate the cell behavior at the level of cell populations or at the level of a single cell. For example, one can create regions with or without cell colonization on a biomaterial surface (Mikulíková et al., 2005; Rezek et al., 2009), or regions selectively colonized with two or more different cell types due to different chemistry or functionalization of the microdomains (Kang and Zhang, 2000). On smaller adhesive microdomains restricted for a single cell, the cell behavior can be regulated by the size and shape of the cell adhesion area (Goessl et al., 2001; Peng et al., 2011; review by Bacakova et al., 2011). On nanopatterned surfaces, the cell behavior can be regulated at subcellular level (Arnold et al., 2008; Coyer et al., 2012; Huang et al., 2009).

This review is focused on polymeric materials modified by plasma discharge, laser irradiation and/or by subsequent chemicals grafting, and on subsequent changes in the material surface chemistry, polarity and charge, wettability, electrical conductivity, morphology and optical properties. The polymers activated by plasma and laser treatment were also grafted with various biologically active molecules and nanoparticles. The latter one provides the material with an additional nanostructure and also with antimicrobial properties (in the case of gold and silver nanoparticles). Special attention was paid to micropatterning and nanopatterning of the material surface. Finally, the newly created material surface properties were correlated with the adhesion, growth and viability of cells, usually of vascular origin.

2. Plasma, ion and laser treatment of materials

The biointerface or biocompatible surface refers to the relationship between synthetic or natural solid state material (polymer or other) and its interaction with tissue, microorganism, cell, virus, or biomolecule (Bauer et al., 2013). Latest morphology research brings a new insight into a wide area of interests from micro-electromechanical systems to data storage devices (ultra-smooth surfaces) to biological and bio-inspired systems, such as microfibers mimicking contacts of insects and geckos with very smooth surfaces (Prokopovich and Rahnejat, 2010). The chemical structure of surface, morphology (Feng et al., 2011), surface chemistry or biological composition of these interfaces, together with surface pattern (distribution and dimensions of the pattern, ranging from the macro- through the micro- to the nano-scale (Hoa et al., 2009; Vasudevan et al., 2014; Pazokian et al., 2011) has ability to strongly influence the behavior, biocompatibility and response of the surface. The mechanism of the surface nanopatterning and the interfacial processes leading to surface structure with demanding parameters are of great importance, together with the development of techniques for the construction of precisely designed surfaces with the aim of direct control and biological response to such modified material (see Fig. 1). (Goddard and Hotchkiss, 2007).

The application of the material science knowledge in fields such as biology, biotechnology, drug delivery or tissue engineering is of great importance in present material research. The key aspect for biomaterial application is the choice of surface treatment method for further material modification, i.e. with appropriate bulk properties (e.g. chemical composition — presence of benzene rings, stability, biodegradable materials) (Tian et al., 2012) or surface functionalities which are able of demanded biological response for a specific application (Fig. 1).

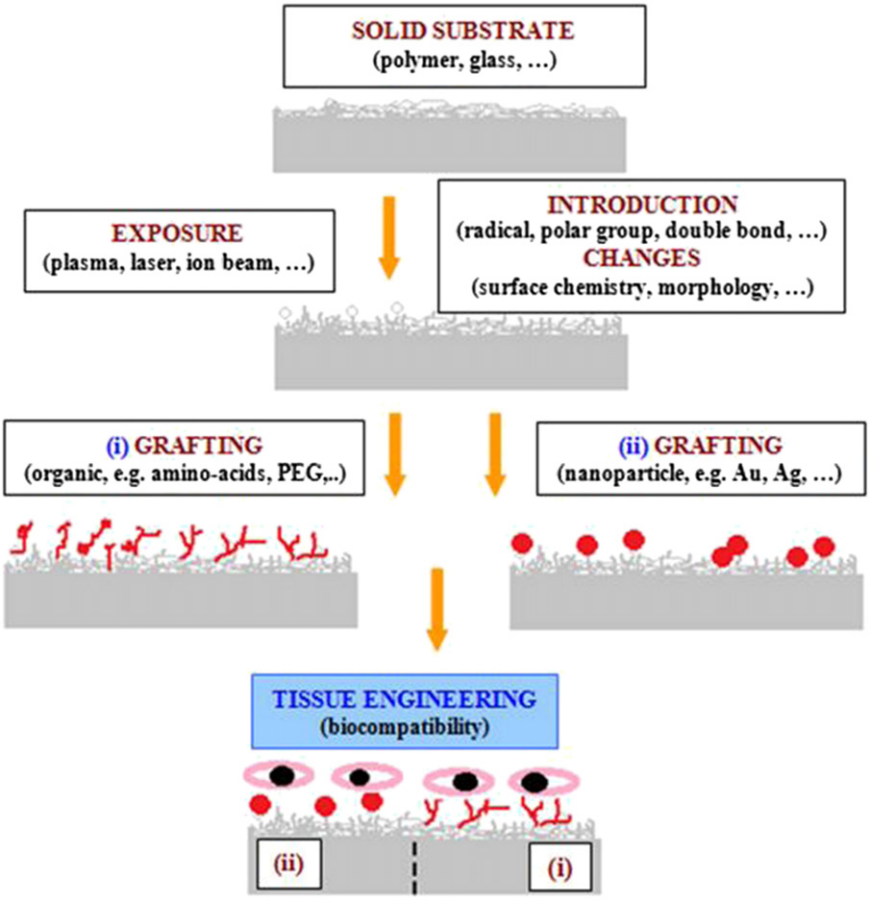

Fig. 1. Scheme of modification of material surfaces and grafting procedures, as a consequence of which the tests of adhesion and proliferation of cells (biocompatibility) are performed.

Different types of biomaterials may be used, i.e. simple polyolefins (Kasálková et al., 2010; Slepička et al., 2012a; Řezníčková et al., 2011), biodegradable polymers (Novák et al., 2012; Synytsya et al., 2009, 2012) or materials based on special additional treatments (Grausova et al., 2009; Vandrovcova et al., 2008; Pařízek et al., 2013).

Patterning techniques (applicable for biocompatibility enhancement) can be divided into two main groups, in general. Top-down patterning approach uses large instruments to create smaller features on a selected substrate (Li et al., 2011). This approach requires rather expensive instrumentation (Rebollar et al., 2008), the quality of which may define the resolution achieved. The geometrical result (the selected pattern) may be also modified with specific software in the case of selected processing techniques. As an alternative method for substrate's surface modification, simple method of surface roughness and surface morphology alternation can be applied. This method is based either on simple modification technique by plasma, which can be further enhanced with post-treatment heating procedures or the direct laser processing under different angles can be applied (Slepička et al., 2011). The laser processing techniques can be either used for regular pattern formation or just as a simple method of polymer surface treatment. Both of these methods can be further developed and subsequent processing techniques can be applied on such activated surface (e.g. metal deposition) (Siegel et al., 2010). The surface treatment which leads to regular surface pattern can be applied only on such polymeric material, which is able to absorb the laser light of defined wavelength thus changing the gradient of heat flow on the modified surface (Bäuerle, 2000). The process is rather complicated (Bäuerle, 2000) and was described several times either applicable for metal nanowire construction (Siegel et al., 2013a) or for cell growth scaffold (Rebollar et al., 2008). The treatment procedure induces changes of surface chemistry, wettability and morphology of polymer surface (Kasálková et al., 2010; Slepička et al., 2012a; Kotál et al., 2007). Different types of materials can be used for biocompatibility enhancement, but the common condition has to be fulfilled, the polymer has to be non-toxic itself, and also all of the degradation products have to suit this condition (Qiao and Liu, 2014). The plasma procedures (Slepičková Kasálková et al., 2013) can be also combined with the subsequent heat treatment, which can significantly influence the physico-chemical properties of material and thus its biocompatibility.

If the substrate is biodegradable and biocompatible itself, the modification procedures can significantly alter its surface properties (Morent et al., 2011; Slepička et al., 2013b), but the improvement of the cell adhesion and proliferation of modified substrate may be not significant, such as e.g. for treated PLLA (Slepička et al., 2012b), even the surface morphology is changed significantly (Slepička et al., 2013d). On the contrary, modified polymer with only a minor change in surface morphology (i.e. the polymer exhibits good stability and resistivity to plasma discharge) as was observed for PMP (Slepička et al., 2012a) can significantly enhance its cell affinity after plasma exposure. It was found that plasma modification resulted in minor changes of PMP morphology. The interaction of cell with substrate can be studied either by simple analysis involving the number of adhered or proliferated cell or specific methods for the cell–substrate interaction can be performed (Xiang et al., 2011). The plasma or laser treatment can be used either for the plane polymer modification or selective enhancement of the polymer properties (Akkan et al., 2013; Förch et al., 2005; Sardella et al., 2006). Also fluorocarbon plasma can be successfully used for surface modification (Di Mundo et al., 2010; Kang and Zhang, 2000).

Laser treatment with pattern formation has been successfully applied on several types of polymer surfaces (Lim and Donahue, 2007; Reisinger et al., 2010; Slepička et al., 2013a, 2014). The

treatment can be also performed through a contact mask (Neděla et al., 2014). Polyethersulfone was found to be one of the most favorable polymers for hemodialysis membranes (Barzin et al., 2004). Surface chemistry of biomaterials and their morphology influence many applications both in medicine or biotechnology with aim to influence the cell growth (Peterbauer et al., 2011; Rebollar et al., 2008). The direction of cell's cytoplasmic protrusions can be controlled by the orientation of the ripples. Properties of nanopatterned surface can influence consecutive alignment of various types of proliferated cells (Rebollar et al., 2008). Micropatterning and nanopatterning are used today to obtain not only precise microstructured and nanostructured surfaces, but also biomolecular positioning on the substrate can influence cellular morphology and biointerfacial interaction connected with cellular adhesion, proliferation, differentiation and migration (Koegler et al., 2012) as well as conformation and functionality of individual proteins attached to the surface together with biomolecular manipulation (Hook et al., 2009). Micropatterning and nanopatterning techniques together with advanced microscopic techniques allow the creation of a new level of detail and thus to understand and influence surface characteristics that influence the function of proteins or peptides (Slepičková Kasálková et al., 2014), which may have a positive effect on cellular adhesion or proliferation.

The effect of ion irradiation on polymer structure and composition is a result of complex physical and chemical processes initiated by energy deposited by ion in polymer matrix (Fink, 2004). Since the energy of bond dissociation in polymers does not exceed ca 10 eV, the energy transferred by an ion leads to multiple cleavage of chemical bonds along the ion trajectory and formation of first generation of degradation products exhibiting high chemical reactivity. Relative fractions of these products depend on the polymer initial structure and composition and on the energy deposited by the ions (Davenas et al., 2002). Some of volatile degradation products (mostly light weight, hydrogen rich ones) may diffuse to the polymer surface and escape. This process leads to gradual carbonization of the polymer surface layer and creation of excessive free volumes (Murphy et al., 2004). The ion beam implantation changes only surface layer of polymers, within the thickness in nm–μm range, and preserves favorable bulk properties of polymers. By the ion implantation or plasma treatment surface properties such as chemical structure (Švorčík et al., 2006, 2007), wettability (Švorčík et al., 2006, 2007), electrical conductivity (Bačáková et al., 2001a,b; Švorčík et al., 2000, 2006), tribological properties (Švorčík et al., 1998, 1999), surface morphology (Švorčík et al., 1998) and cytocompatibility (Švorčík et al., 2000) can be changed in a manner which can be controlled by a proper choice of ion mass, energy and fluence. Also implanted atoms may form new structures in the polymer interior with specific electrical, magnetic or optical properties. The polymers modified by the ion implantation may further be affected by exposing them to suitable chemical agents, which may interact with the modified polymer surface and form new functional structures on it.

3. Grafting the polymers with molecules and nanoparticles

The nanostructure of the material surface is thought to mimic the morphology of the cell membrane, and also the architecture of ECM molecules (Webster and Smith, 2005). The modification of polymers in Ar plasma is an important technique both for surface morphology or chemistry modification (Kasálková et al., 2010; Švorčík et al., 2009). The subsequent grafting procedures (e.g. with PEG) can significantly improve cell adhesion and proliferation (Kasálková et al., 2010; Slepička et al., 2012c). The optical microscopy techniques can be used for cell proliferation determination.

Grafting is a process where the functional groups are attached to the polymer by e.g. plasma activation (Kolská et al., 2014; Landgraf et al., 2009; Řezníčková et al., 2012; Slepička et al., 2013c; Slepičková Kasálková et al., 2012) and can significantly affect the cellular response of the surface. Primary adhesion is characterized by non-specific and reversible interactions, which are combinations of non-covalent interactions, i.e., van der Waals, hydrophobic and steric and interactions based on hydrogen bonding (Bos et al., 1999). The status of secondary adhesion is referred to achievement of irreversible cell's capture, which is associated with the development of specific binding interactions between the interacting surfaces (Verstrepen and Klis, 2006). The destruction of cancer cells may be supported by gold nanoparticles (Gannon et al., 2008).

The polymer scaffolds are constructed with aim to serve as "an environment" to enhance cell attachment, migration, proliferation and differentiation (Waterhouse et al., 2012). Also the ECM structure may significantly influence tissue function (Gauvin et al., 2011). Biologically active molecules can be incorporated into scaffold or attached onto polymer surface and thus we can regulate number of cell response (Gauvin et al., 2011). Polyolefins' modification with argon plasma in combination with grafting procedures can affect the polymer's cell colonization (Novotná et al., 2013; Slepičková Kasálková et al., 2014). The behavior of vascular smooth muscle cells (VSMC) on polyolefins can be regulated by the chemistry of grafted bioactive substances (Parizek et al., 2009; Rimpelová et al., 2013). Cysteamine was grafted on different polymer foils (e.g. poly-L-lactic acid (PLLA), polystyrene (PS), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polyethylene terephthalate (PET), polytetrafluoroethylene (PTFE), polyvinyl fluoride (PVF) and polyvinylidene fluoride (PVDF)) previously treated (activated) in plasma discharge (Kolská et al., 2014). It was found that plasma treatment and cysteamine grafting improve dramatically surface cytocompatibility (Kolská et al., 2014).

One of analytical methods to characterize surfaces of tested substrates was an electrokinetic analysis due to this method gives information about real surface chemistry and charge important for cell adhesion. Treatment with plasma and ion beam influences the surface charge, chemistry and polarity, as well as surface zeta potential. The plasma treatment results in scission of original bonds (e.g. C – H, C – C, C – O), and creation of new radical sites on surface ("free" radicals, double bonds and new chemical groups (Švorčík et al., 2001, 2002, 2006; Ročková et al., 2004; Bacakova et al., 2011). More pronounced results are obtained after cysteamine grafting. The most dramatic increase in zeta potential was obtained on non-polar polymers (PTFE and HDPE), while, on most polar polymers (PET and PLLA) the zeta potential remains almost unchanged. Different behaviors of these two groups of polymers may be due to (i) different amount of grafted cysteamine and also (ii) different way of preferential bonds of cysteamine (via – SH or $-NH_2$ group) to surface (Kolská et al., 2014). Comparison of these results with XPS measurement obtained on grafted samples shows the higher amount of concentration of nitrogen on PTFE, PS, PVF, PVDF in comparison with sulfur. This indicates that cysteamine is bonded at these surfaces preferentially via – SH group to the surface and with $-NH_2$ group remaining free. Scheme of cysteamine grafting on different polymers is presented in Fig. 2 and more detailed explanation of this different behavior of grafting cysteamine is described in (Kolská et al., 2014).

In work (Kolská et al., 2014) the VSMC cell response was studied in vitro on the selected samples of PTFE, HDPE, PET and PLLA and compared with TCPS substrate used as a control. It is evident that the plasma treatment or cysteamine grafting significantly improved VSMC's adhesion in comparison with pristine polymers especially on non-polar polymers (HDPE and PTFE). Most dramatic effect is obtained for PTFE plasma treated and grafted with cysteamine which is even comparable with the control TCPS (Kolská et al., 2014). The preferential orientation of grafted cysteamine on PTFE, PS, PVF and PVDF leads to more positive surface (due to presence of $-NH_2$ groups), which is attractive for cell adhesion and response. It is known that the major proteins (especially proteins of fetal bovine serum) as well as cell membranes are negatively charged under

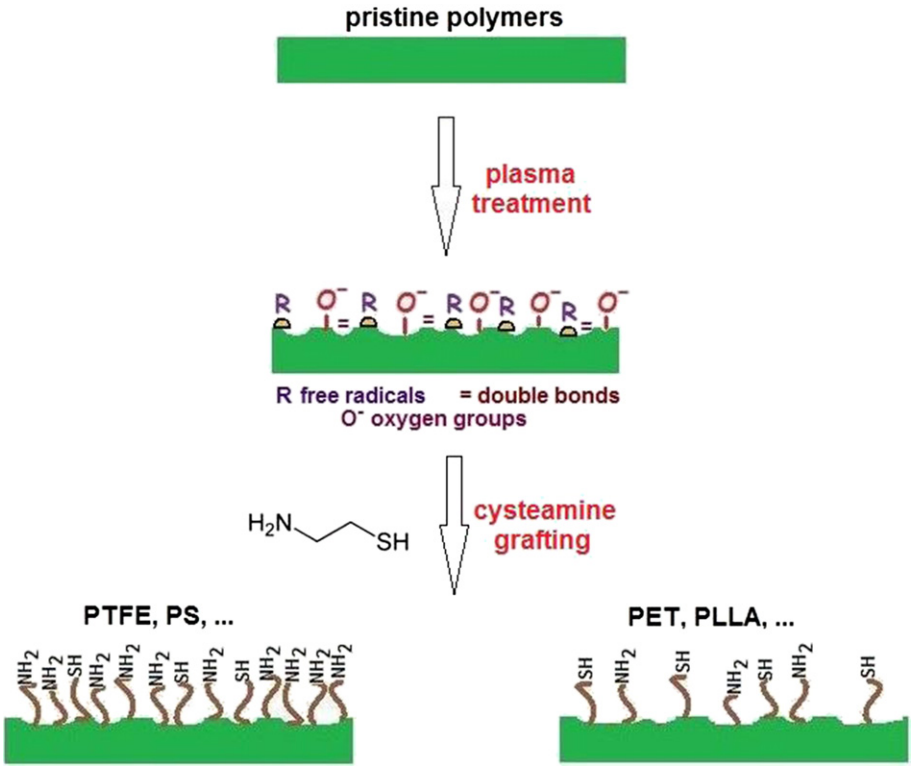

Fig. 2. Scheme of cysteamine grafting on plasma treated polymers (Kolská et al., 2014).

physiological pH. The adhesion of cells with negatively charged membranes may be facilitated by electrostatic interactions and the better cell adhesion may be expected on positively charged surfaces. Results obtained in our work (Kolská et al., 2014) are similar to those obtained previously for human umbilical vein endothelial cells (Arima and Iwata, 2007) or for human fibroblasts (Faucheux et al., 2004) on surfaces modified by amino groups.

The alternative approach to implement new functionalities and thus create nanostructured surfaces is grafting of the nanoparticles on polymer surfaces. The different uptake behaviors for surface modified nanoparticles can be explained for the different charge of nanoparticles and cell membrane (Mailänder and Landfester, 2009). Au nanoparticles are also used for diagnosis and even destruction of microorganisms for biological applications (Reznickova et al., 2013) or as biosensors.

Carbon nanotubes (CNT), diamond like carbon (DLC) and carbon nanodiamonds (CND) are the most studied carbon nanostructures (CNPs) for biomedical applications. Diverse types of CNT are studied in tissue engineering for their electrical properties as scaffolds for

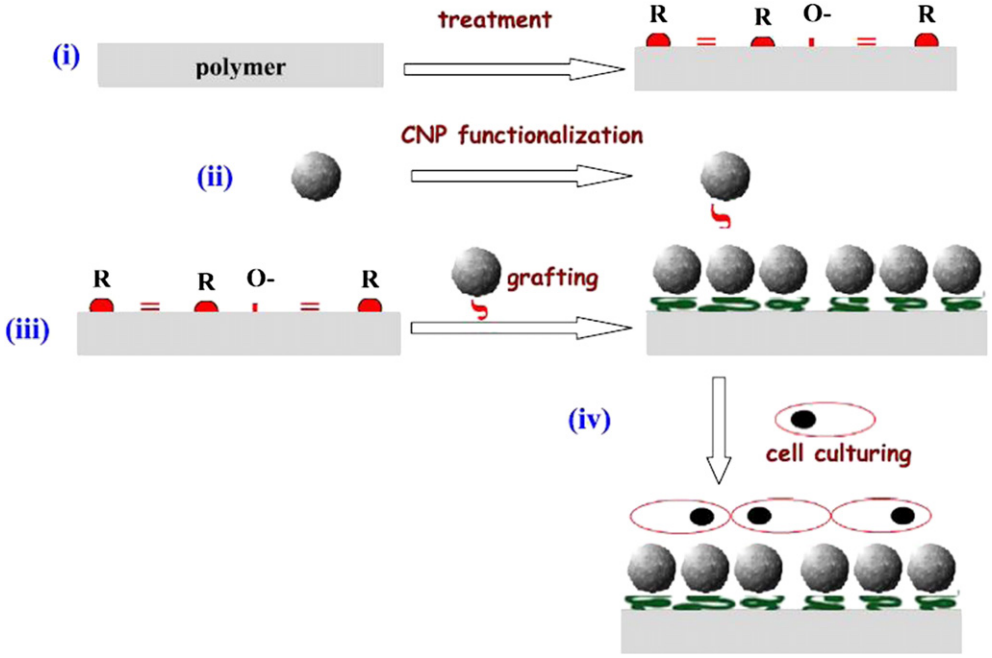

Fig. 3. Scheme of grafting of functionalized carbon nanoparticles (CNPs): (i) plasma treatment (activation) of polymer, (ii) functionalization of CNPs, (iii) grafting of modified CNPs on plasma activated polymer and (iv) cell adhesion and proliferation (Švorčík et al., 2014).

treating severed nerves (Tavangarian and Li, 2012) or as reinforcements for composites with polymer substrates for bone replacement in orthopedics (Liao et al., 2013). Functionalization of carbon nano-particles with nitrogen (amine) groups, grafting of plasma treated PET and HDPE with functionalized CNPs and the effects of such modifications on polymer biocompatibility were also studied (Švorčík et al., 2014). The scheme of the plasma activation and CNPs functionalization is shown in Fig. 3. The functionalization procedure CNPs was adjusted to avoid a "saturation" of amino groups on the CNPs which was observed in the work (Trostová et al., 2013) when the "small" functional group concentrates in CNPs pores. It is evident that due to the functionalization of CNPs with amino groups, the preferential bonding was realized (Švorčík et al., 2014). It can be assumed that amino groups react with carboxyls as $R\text{-}NH_3^+ + {}^-OOC\text{-}R$ (i) on the plasma treated surface of polymers. Because of the acidic environment, the opposite interaction cannot be excluded, i.e. $R\text{-}CO\text{-}OH + NH_2\text{-}R$ (ii), although it is less likely than the reaction (i). Furthermore, it can be assumed that carbonyl groups on the plasma treated surface may also participate in the grafting process, therefore the imine can be created, $RR\text{-}C{=}O + NH_2\text{-}R \rightarrow RR\text{-}C{=}NH$ (iii). The bonding of amine on radical sites created by the plasma treatment cannot be excluded too (Švorčík et al., 2014).

Carbon grafting and plasma modification also significantly influence the surface morphology, the successful grafting procedure was confirmed by AFM measurement in phase mode (Švorčík et al., 2014). According to the AFM phase analysis it was confirmed that at least 30% of the surface is chemically different, which can be evaluated as a proof of the CNP's presence. Cellular response of VSMCs was studied in vitro on the CNP grafted polymers (Švorčík et al., 2014). No dramatic differences in surface structure of CNPs before and after the functionalization with amino groups are seen on TEM images. After the interaction of plasma activated polymer surface with functionalized CNPs an increase in nitrogen concentration was observed. It was proposed, that amino groups react with carboxyls produced by the plasma treatment (ionic bond) but participation of carbonyl groups and radicals cannot be excluded too. The grafting of polymers with activated CNPs influenced positively a cellular response of VSMC cells. This finding may be of interest for application of the modified polymers in tissue engineering.

4. Antibacterial properties of gold and silver nanoparticles sputtered into liquid

In this section a brief overview on results published in the field of metal nanoparticle synthesis and investigation of their antibacterial action is introduced. When applying metal nanoparticles into living tissues, fundamental attention must be paid to the NP synthesis process itself, especially for anti-microbial applications (Rai et al., 2009) For non-invasive application of metal nanoparticles into living microorganism our group has developed novel method of direct sputtering of metal into liquid glycerol (Siegel et al., 2012). Considering this, direct metal deposition into the glycerol seems to be promising technique combining the advantages of non-toxic and environmentally friendly process completely omitting the usage of solvents or reduction agents compared to classical wet-based methods. Furthermore, the possibility of tailoring the nanoparticle size via controlling the temperature of capturing media as well as the investigation of the functions of NP size and metal type (silver and gold) on the antibacterial activity is demonstrated. The antibacterial properties of these NPs were tested against two common pollutants of *E. coli* (Gram-negative bacteria) and *Staphylococcus epidermidis* (*S. epidermidis*, Gram-positive bacteria) naturally occurring on the skin and mucous membranes of human. Prepared NPs were studied by TEM and HRTEM and UV–vis spectroscopy (Siegel et al., 2012). TEM images revealed that size of Au and Ag NP nanoparticles did not exceed 5 nm (Siegel et al., 2012). Stability of nanoparticles was determined to be propane-1,2,3-triol (glycerol)/H_2O ratio dependent. It was earlier confirmed, that glycerol –OH group can stabilize AgNP's suspension by its adsorption onto particles (Sarkar et al., 2010). However, the process is not yet fully understood.

Targeted variation of metal nanoparticle size belongs to great challenges of current science. Change of capturing media temperature may provide broad spectrum of nanoparticle diameters. When applied this technique, both $AgNP_{4-6}$ and $AuNP_{4-6}$ (indexes refer to average nanoparticle size) exhibit distinctive narrow peaks with maximum absorption at 400 and 520 nm, respectively (Siegel et al., 2013b). Measured spectra are almost identical to those obtained in our former study on Ag and Au with average diameter of 3–5 nm indicating stable solutions with minimal particle dispersion. Considerable broadening with pronounced shift of absorption peek occurs at $AuNP_{9-12}$. This phenomenon originates from increase in dimensions of individual Au particles and broader size distribution compared to $AuNP_{4-6}$. Distinctive red coloration of $AuNP_{4-6}$ turns into purple one, belonging to $AuNP_{9-12}$, as the average size increases from ca 5 to 10 nm.

The growth of *E. coli* and *S. epidermidis* was completely inhibited in the presence of $AgNP_{4-6}$ after 24 h (for both 6 and 24 h incubated samples) when compared to the control samples (bacteria incubated in glycerol or physiological saline solution [PBS]), see Table 1 (Siegel et al., 2013b). The growth inhibition of both bacterial strains was further maintained even after 30 and 48 h of growth, suggesting strong bactericidal activity of $AgNP_{4-6}$. Surprisingly, $AuNP_{4-6}$, but not $AuNP_{9-12}$, was able to inhibit the growth of *S. epidermidis* and the effect was preserved for whole tested period (48 h), when compared to control samples. The antibacterial action of Ag^+ ions has been broadly reported so far (Klaus et al., 1999), also AgNPs repeatedly showed their potency against bacteria (Raffi et al., 2008). Thus, our results (growth inhibition of both bacterial strains by $AgNP_{4-6}$) are in agreement with other groups results. Biocidal properties of AuNPs of similar size (5 nm) as prepared by our group (4–6 nm), were observed by Lima et al. (Lima et al., 2013). Their AuNPs dispersed on zeolites were effective against Gram-negative *E. coli* and *Salmonella typhi* (90–95 % growth inhibition). It has been reported, that the antibacterial response of AgNP depends on particle size and shape (Pal et al., 2007; Panáček et al., 2006). Thus, it is very likely that also the size of AuNP plays significant role in the antimicrobial action.

Above results support the hypothesis (Siegel et al., 2013b) that Ag and Au nanoparticles can be prepared in a simple and cost-effective manner and are usable for construction of new types of antibactericidal substrates. The method is based on temperature control of capturing media during the sputtering deposition process into glycerol. In this way the size of Au nanoparticles can be varied from ca 4 to 12 nm. Bactericidal action of such synthesized NPs was examined against two common pollutants (*E. coli* and *S. epidermis*). While $AgNP_{4-6}$ completely inhibited both bacteria strains after 24 h, AuNPs exhibited pronounced inhibition selectivity regarding to the specific NP dimensions. Regardless of AuNP size, any growth inhibition of *E. coli* occurred during the whole experiment. Contrary to that, $AuNP_{4-6}$ was able to inhibit the growth of *S. epidermidis*.

Table 1
Inhibitory effect of silver and gold nanoparticles against Gram-negative (*E. coli*) and Gram-positive (*S. epidermidis*) bacteria ([a]time length of bacterial growth on LB plates after inoculation, [b]an empty circle indicate inhibition effect, [c]a full circle indicate positive growth, [d]physiological saline solution, [e]a half full circle indicate 50% growth) (Siegel et al., 2013b).

Bacteria	*E. coli*			*S. epidermidis*		
Growth time (h)[a]	24	30	48	24	30	48
$Ag\ NP_{4-6}$	○[b]	○	○	○	○	○
$Au\ NP_{4-6}$	●[c]	●	●	○	○	○
$Au\ NP_{9-12}$	●	●	●	○	●	●
Glycerol	●	●	●	○	●	●
PBS[d]	●	●	●	◗[e]	●	●

5. Conclusion

Polymeric materials are important cell carriers for tissue engineering. However, in their natural form they did not often possess the properties which are suitable for their application as biocompatible substrates. A special issue is the micropatterning or nanopatterning of the polymer surface. The reason is to create microdomains or nanodomains with different properties (most frequently with domains of different wettability, with periodically arranged depressions and prominences). The micropatterned surfaces are able to regulate the cell behavior at the level of cell populations or at the level of a single cell. The successful method for surface treatment which can be further combined with grafting procedures is plasma treatment. The process allows the change of the relationship between synthetic or natural solid state material (polymer or other) and its interaction with tissue, microorganism, cell or biomolecule. Subsequent grafting procedures are of great importance either for biocompatibility improvement or update of material's antimicrobial properties. Carbon or gold nanoparticles, or bioactive molecules like cysteamine can be grafted on polymer surfaces activated by plasma and thus significantly enhance its biocompatible properties. New methods of anti-bacterial substances construction were proposed. The function of polymers as advanced cell carriers can be significantly enhanced which allows the regulation of cell–material interaction and antimicrobial activity of selected substrates.

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