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Review article

Biomimetic polymers in pharmaceutical and biomedical sciences

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Abstract

This review describes recent developments in the emerging field of biomimetic polymeric biomaterials, which signal to cells via biologically active entities. The described biological effects are, in contrast to many other known interactions, receptor mediated and therefore very specific for certain cell types. As an introduction into this field, first some biological principles are illustrated such as cell attachment, cytokine signaling and endocytosis, which are some of the mechanisms used to control cells with biomimetic polymers. The next topics are then the basic design rules for the creation of biomimetic materials. Here, the major emphasis is on polymers that are assembled in separate building blocks, meaning that the biologically active entity is attached to the polymer in a separate chemical reaction. In that respect, first individual chemical standard reactions that may be used for this step are briefly reviewed. In the following chapter, the emphasis is on polymer types that have been used for the development of several biomimetic materials. There is, thereby, a delineation made between materials that are processed to devices exceeding cellular dimensions and materials predominantly used for the assembly of nanostructures. Finally, we give a few current examples for applications in which biomimetic polymers have been applied to achieve a better biomaterial performance.

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1. Introduction

Besides the well-known application of low-molecular weight substances, like drugs, the application of bigger non-drug materials—like polymers, ceramics or metals—to the human body is valuable to treat, enhance, or replace a damaged tissue, organ, or organ function. Originating from their application in the biological environment, these materials are called biomaterials, because of their ability to replace or restore biological functions and exhibit a pronounced compatibility with the biological environment [1,2].

Biomaterials in general have been used for numerous applications in which their contact to cells and tissues via their surface is of utmost importance. Apart from their original use as a tissue replacement, they have increasingly been applied as carriers for drugs [3] and cells [4–8] in recent years. The characterization of the material interaction with cells was, thereby, frequently concentrated on issues such as biocompatibility [9–12], initiation of tissue ingrowth into the material's void space or host tissue integration. Although these properties are of paramount significance for biomaterial development and application, cell/material interactions have primarily been considered on a generalized scale, as the underlying mechanisms remain widely elusive due to the complexity and multitude of parameters involved. While research along these traditional lines has resulted in a number of biomaterials with significantly improved properties, the question arose in recent years if one could not take better advantage of biology's potential to interact with its environment more specifically. Doing so would facilitate the development of biomaterials for applications that require the control of cell behavior with respect to individual processes such as cell proliferation [13,14], cell differentiation and cell motility [15–18]. In an ideal case, this would allow for the 'design' of a material to elicit cellular responses that help the material

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to better perform its intended task. Applications for such designer-materials range from tissue repair or replacement to the controlled cellular uptake for the delivery of therapeutic agents [19,20].

There are two major categories of cell–biomaterial interactions: specific and unspecific. Unspecific interactions are usually difficult to control, because they are based on properties common to multiple cell types. These common cell characteristics include, for example, cell surface properties, such as the negative charge of the cell membrane, as well as ubiquitous lipophilic membrane proteins or lipophilic proteins of the extracellular matrix (ECM) that mediate unspecific adhesion to polymer surfaces.

Specific interactions, in contrast, are much more controllable as they are primarily related to the interactions of defined chemical structures, such as ligands that interact with their corresponding cell surface receptors [21]. The expressions 'biomimetic' and 'bioactive' have been coined to describe materials that are capable of such defined interactions [22,23]. In particular, biomimetic materials are materials that mimic a biological environment to elicit a desired cellular response, facilitating the fulfillment of their task [24,25]. It is obvious that drugs do not fit into such a definition, as their task is the interaction with cells 'per se'. Biomaterials of a natural origin also do not unequivocally fit into this category, because they do not mimic a natural environment, but rather provide one. Despite these crisp definitions, a gray zone exists in which materials cannot explicitly be classified.

So what is the blueprint of a biomimetic material after all? It is obvious that, for example, receptor ligands integrated into the material play an important role with respect to cell–material interactions. One has to bear in mind that the main task of a biomimetic material is not necessarily the specific interaction with a cell or tissue, but rather the fulfillment of the intended purpose, e.g. the targeting of a certain cell type or providing a scaffold structure for tissue growth; this specific interaction is intended as a tool for the material to achieve these goals. One of the first types of biomimetic materials targeted the integrin receptor to enhance cell adhesion to material surfaces [26,27]. Such materials contained exposed RGD motifs on their surface [28,29]. Other materials had cytokines tethered to their surface to target cell surface receptors that impact cell proliferation or differentiation [13,14,18]. Some of these materials have been extraordinarily successful and it is expected that more and more biomaterials will be developed that mimic the properties of biological environments in order to influence cells and whole tissues.

It is the goal of this review to give an overview of the field of biomimetic materials, which is scattered among different disciplines, such as biomaterials science, biomedical engineering, the medical sciences and pharmaceutics. It is obvious that the definitions given above include a variety of material design principles and a number of material classes. Mimicking a natural environment could, for example, also be a matter of shaping a material on the micrometer and nanometer scale, dimensions that cells can 'sense' and respond to in defined way [30–32]. As a treating of the whole field is beyond the scope of this single paper, we will focus exclusively on materials that interact with cells via receptors. In the first chapter, we will elucidate the mechanisms by which cells can interact with their environment, which provide the basis for a rational material design. In the following chapter, we will review the chemistry by which cell surface receptor ligands can be attached to the materials. Next we will consider two limiting cases: the scenario in which the dimension of the biomimetic material vastly exceeds the dimensions of a cell and the reverse case in which the cell is much larger than the material, which is then essentially in the nanoscale. In both cases, we report on the particular aspects of material design and actual developments. Finally, we review potential applications of biomimetic materials in tissue engineering, polymer-associated drug targeting and non-viral gene transfer into mammalian cells.

2. Mechanisms by which cells can interact with their environment

Mechanisms of cellular interaction with the environment are of paramount significance for biomimetic material development. In vertebrate tissues, many mechanisms exist that enable cells to communicate with their environment, specifically by means of signaling molecules. The principle of this interaction is that a ligand binds to its corresponding receptor leading to various intra- and extracellular responses. In this chapter, we will elucidate the biological principles of three interactions that are of interest for biomimetic material design: cell adhesion, morphogenic stimuli signaling and endocytosis.

2.1. Cell adhesion

Cell adhesion is a critical process in the field of biomaterials. In tissue engineering, for example, cell attachment is an obvious prerequisite for a number of important processes, such as cell proliferation or cell migration [33], but cell adhesion is an important component even for more established biomaterial applications such as orthopedic implants [34]. However, in many applications it may be crucial to ensure the adhesion of specific cell types. Therefore, a tremendous amount of research has been devoted to understand and, consequently, control cell adhesion.

2.1.1. Integrin-binding peptides

Cell–matrix adherens junctions enable cells to bind the ECM by connecting the actin filaments of their cytoskeleton

to the matrix. Members of a large family of cell–surface matrix receptors called *integrins* mediate this adhesion. Integrins are composed of two non-covalently associated transmembrane glycoprotein subunits (α and β). 18α- and 8β-Units have already been discovered, which form 24 known different heterodimers [35].

The tripeptide sequence Arg-Gly-Asp (RGD) has been identified as part of many natural integrin ligands and a motif on several ECM proteins [36]. The variety of receptors with different α and β subunit combinations gives rise to differences in the receptor affinity of different RGD containing compounds. Many small adhesion peptides (RGD peptides) have been synthesized, for example RGD, YRGDS, CGRGDSY, as well as cyclic RGD peptides such as cyclo(RGDfK) [27]. About half of the 24 known integrin receptors bind to ECM molecules in a RGD dependent manner [37]. Due to the fact that integrins are distributed and used throughout the organism, the RGD sequence is an attractive compound to utilize in the stimulation of cell adhesion on synthetic surfaces.

Cell adhesion involves a sequence of four steps: cell attachment, cell spreading, organization of an actin cytoskeleton, and formation of focal adhesions (Fig. 1) [28,38]. Following cell attachment, cells are sufficiently associated with the material to withstand gentle shear forces, whereas during the second phase the cell body becomes flat and its plasma membrane spreads over the substratum. Thereafter, actin organizes into microfilament bundles that form an actin cytoskeleton. A forth effect is the formation of focal adhesions that link the ECM to the actin cytoskeleton. A great number of signaling events following the formation of focal adhesions are known [39].

2.1.2. Heparin-binding peptides and lectins

Among the non-integrin surface receptors, proteoglycans, such as the syndecans [40], constitute a large family of molecules responsible for cell adhesion. They consist of a core protein to which the negatively charged glycosaminoglycan is covalently attached [28]. Therefore, the heparin-binding domains are rich in basic amino acids and numerous heparin binding sequences based on X-B-B-X-B-X or X-B-B-B-X-X-B-X structures have been identified [41], where B represents a basic amino acid and X a hydropathic residue. KRSR, for example, was selectively used to promote osteoblast adhesion [42]. However, cell attachment using these sequences is usually less significant compared to integrin-binding RGD.

The carbohydrate-rich zone on the cell surface, known as the glycocalix, can be characterized by its affinity for carbohydrate-binding proteins called lectins [43]. Wheat germ agglutinin (WGA), for example, recognizes these carbohydrates and can therefore be used for targeting cells [44].

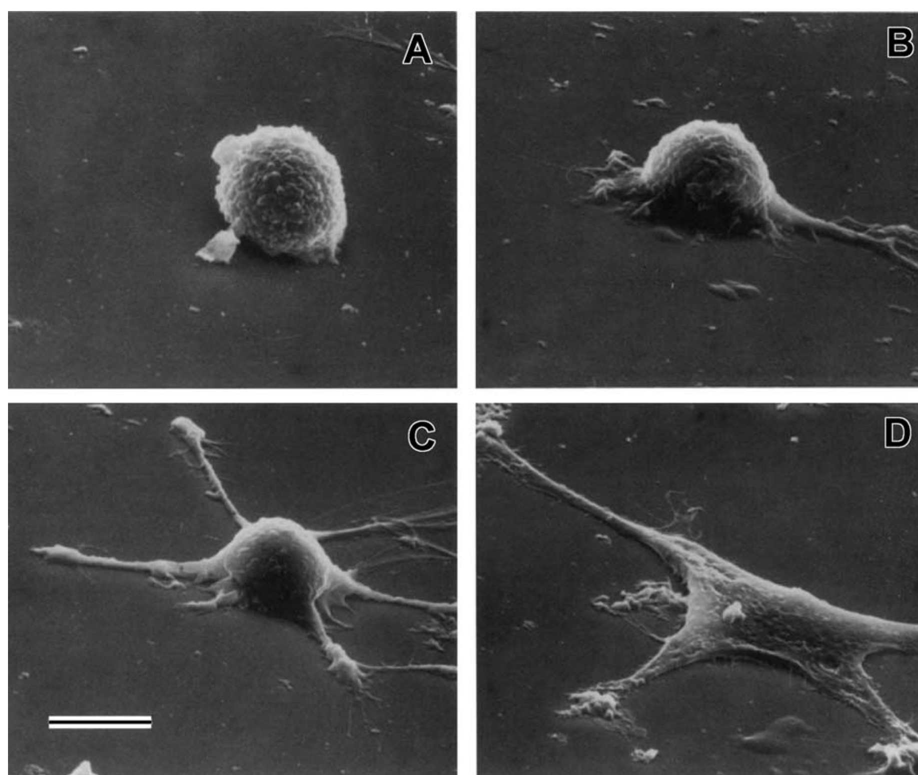


Fig. 1. Process of cell attachment to cell spreading. Scanning electron micrographs of adherent cells on substrates containing varying concentrations of covalently grafted peptide (GRGDY). (A) Spheroid cells with no filapodial extensions; (B) spheroid cells with one to two filapodial extensions; (C) spheroid cells with greater than two filapodial extensions; (D) flattened morphology representative of well spread cells. Bar: 10 μm. Reproduced from The Journal of Cell Biology, 1991, vol. 114, pp. 1089 by copyright permission of The Rockefeller University Press [38].

2.2. Morphogenic and mitogenic factor signaling

While the aforementioned mechanisms of communication were linked to the attachment of cells, morphogenic and mitogenic factors affect other processes such as cell mobility, cell differentiation cell proliferation. Growth factors are a class of bioactive molecules that hold great potential the development of biomimetic polymers. These polypeptides manage cellular activities through a complex network of intracellular signaling cascades. They engage in processes such as cellular proliferation, differentiation, migration, adhesion and gene expression. For each type of growth factor, there is a specific receptor or set of receptors, which some cells express on their surface and others do not.

The receptors for most growth and differentiation factors are a large family of transmembrane tyrosine protein kinases. They include receptors for vascular endothelial growth factor (VEGF), fibroblast growth factors (FGFs), epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I) and many others.

VEGF is, amongst other functions, the key regulator of normal and abnormal angiogenesis, a specific mitogen for vascular endothelial cells derived from arteries, veins, or lymphatics [45] and therefore used as a promising candidate for the stimulation of angiogenesis-dependent tissue regeneration.

FGFs are polypeptide growth factors that initiate mitogenic, chemotactic and angiogenic activity [46]. Some FGFs are potent angiogenic factors and most of them play important roles in embryonic development and wound healing. In contrast to VEGF, FGFs are pleiotropic, i.e. they control distinct and seemingly unrelated effects, because they stimulate endothelial cells, smooth muscle cells, fibroblasts and certain epithelial cells [47].

EGF exhibits mitogenic and motogenic activities [48,49] and is present in many cell types, including fibroblasts and epithelial cells. EGF, in addition to transforming growth factor-α (TGF-α), is thought to be an important factor in inflammation and wound healing by stimulating neovascularization and chemotaxis of cells involved in wound healing [50].

IGF-I has successfully been shown to induce proliferation of chondrocytes and stimulate the synthesis of ECM components in an in vitro cartilage model [51]. Furthermore, it has been demonstrated that the IGF-I receptor is different from the insulin receptor, but there is communication between IGF-I and insulin and their receptors [52].

The TGF-β superfamily comprises a large number of polypeptide growth factors [53] and, in contrast to the above-mentioned factors, they activate receptors that are serine/threonine protein kinases [54]. TGF-β has been shown to play a major role in wound healing and fibrosis, and has been recognized to be very important in tissue repair due to its ability to stimulate cells to deposit ECM [55]. TGF-β1, for example, is a key factor during bone development and regeneration [56,57].

A number of other extracellular signaling proteins are structurally related to the TGF-βs and also belong to the TGF-β superfamily. Among them, the bone morphogenic proteins (BMPs) play an important role in bone formation [54]. BMP-2 is reported to be a useful growth factor to increase osteoblastic differentiation of rat marrow stromal cells (rMSCs) [58,59].

2.3. Endocytosis

A third important biological principle is the particle uptake into cells via lipid bilayer vesicles formed from the plasma membrane, usually termed endocytosis. Being able to activate this mechanism using a biomimetic material would provide tremendous opportunities for delivering drugs and DNA more efficiently into the cell. Two main types of endocytosis are distinguished, generally classified as phagocytosis and pinocytosis. Phagocytosis involves the internalization of large particles (>0.5 μm), whereas pinocytosis describes the formation of smaller vesicles (<0.2 μm) [60]. These vesicles are initiated at specialized regions of the plasma membrane called clathrin-coated pits, which, in association with transmembrane receptors, can serve as a concentrating device for the internalization of specific extracellular macromolecules, a process called receptor-mediated endocytosis. The macromolecules bind to complementary cell-surface receptors, accumulate in clathrin-coated pits and enter the cell in clathrin-coated vesicles that end up in endosomes. Thereafter, the receptor proteins can be recycled, degraded in lysosomes or return a different plasma domain [61].

This process can be used for the uptake of molecules in hepatocytes, which express the asialoglycoprotein receptor (ASGPr), a receptor that selectively recognizes glycoproteins containing galactose residues [62]. The transport of macromolecules into the cell by receptor-mediated endocytosis with transferrin as a targeting moiety via the transferrin receptor can be utilized in rapidly dividing tissues [63]. Another possible uptake route to clathrin-mediated endocytosis is via caveolae [64].

3. Conjugation chemistry for biomimetic molecules

Biomimetic materials can be synthesized in numerous ways. One method includes a complete de novo synthesis of all components including the cell signaling entities. As this is different for each individual material, it is beyond the scope of this review to go into such details. An alternative is the design of the material that can be assembled from components. Molecules that are used for cell signaling are then considered one building block that is attached to the backbone of the material via functional groups on the polymer. This design strategy has the advantage that bioactive molecules can be bound to the material surface after processing the polymer into its final form. In this

chapter we will review the most popular binding reactions that can be used for such an assembly.

3.1. Carbodiimide-conjugation

Carbodiimides belong to the zero length cross-linking agents, forming bonds without the introduction of additional atoms or spacers. Their application is favorable in conjugation reactions, where such spacer might be detrimental for the intended use of the corresponding conjugates. Their applicability in both organic and aqueous solvents contributes to the wide spectrum of possible conjugation reactions (Table 1a).

Carbodiimides are widely used to activate carboxylate groups by the formation of highly reactive O-acylisourea intermediates [65]. This active species can then react with amine nucleophiles to form stable amide bonds. Water soluble carbodiimides, such as 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), allow for an aqueous conjugation reaction of water soluble targeting

Table 1
Conjugation mechanisms

(a) Carbodiimide mediated reaction of amines with carbonic acids. (b) Reductive amination. (c) Reaction of isothiocyanates with nucleophils. (d) Reaction of maleimides with thiols. (e) SPDP mediated crosslinking of amines with thiols. (f) Biotinylation of amines.

molecules and polymers. To circumvent hydrolysis [66], organic soluble carbodiimides, like dicyclohexyl carbodiimide (DCC), have been used to form ester linkages or amides with the corresponding carboxylic acids at high efficacy in anhydrous solutions [67,68]. To avoid undesirable side reactions [69], N-hydroxysuccinimide (NHS) or N-hydroxysulfosuccinimide (Sulfo-NHS) can be added to form more stable NHS ester derivatives as reactive acylating agents. The corresponding NHS or Sulfo-NHS esters react readily with nucleophiles to form the acylated product, but only primary or secondary amines form stable amid or imide linkages, respectively [70]. Many examples of carbodiimide mediated conjugations are present in the literature: the T101-antibody has been directly conjugated to poly
(L-lysine) (PLL) taking advantage of the water solubility of EDC [71], folic acid was covalently bound to poly(aminopoly(ethylene glycol)cyanoacrylate-*co*-hexadecyl cyanoacrylate) using DCC/NHS mediated amide synthesis [72] and, in a different reference, folic acid was also linked to the terminal hydroxyl of the poly(ethylene glycol) (PEG) block of poly(L-histidine)-*co*-poly(ethylene glycol) by DCC and 4-dimethylaminopyridine (DMAP) mediated acylation [73].

3.2. Reductive amination

Reductive amination results in a zero-length cross-linking between aldehyde and amine components, forming stable amine bonds without the introducing of additional, possibly unfavorable spacer (Table 1b).

Native carbohydrates contain aldehyde groups as reducing ends and can be directly coupled with amine-containing molecules, leading to the formation of a Schiff base intermediate. Unfortunately, the direct coupling of the reducing carbohydrates with amines suffers a rather low efficiency, due to the comparatively low concentration of the open structure in aqueous solution compared to the cyclic hemiacetal form. Alternatively, carbohydrates often also contain hydroxyl groups on adjacent carbon atoms, which can be oxidized to reactive aldehyde groups using sodium periodate [74,75].

After the reaction in an aqueous environment, Schiff bases are rapidly reversed to the corresponding aldehyde and amine by hydrolysis. The Schiff bases formed can be converted into stable secondary amine linkages by reductive amination using reducing agents, such as sodium cyanoborohybrid, which reduces Schiff bases efficiently while aldehydes do not react [76,77]. Carbohydrates like galactose have been directly coupled to polyethylenimine (PEI) by reductive amination [78], while transferrin, a glycoprotein, was oxidized using the periodate oxidation method before conjugation with the amine component PLL [79].

3.3. Isothiocyanate reaction with nucleophiles

Isothiocyanates are homobifunctional linkers, which react almost selectively with primary amines leading to the formation of stable thiourea compounds. Unfortunately, their use is afflicted with only poorly controllable reactions, the formation of rather random conjugates, as well as polymerization or intramolecular cross-linking giving byproducts with altered solubility (Table 1c).

The reaction has its pH optimum at an alkaline pH, where amines are deprotonated [80]. With the help of the isothiocyanate linker, galactose and lactose have been conjugated to PLL [81].

3.4. Reaction of maleimides with sulfhydryls (thiols)

Maleic acid imides (maleimides) are also an integral part of many heterobifunctional cross-linking agents, allowing for the covalent attachment of bioactive molecules to polymers in a two-step procedure. This minimizes the side reactions prevalent in the use of homobifunctional linkers. Over a pH range of 6.5–7.5, maleimides can be specifically alkylated at their double bond by a reaction with sulfhydryl (thiol) groups to form thioether bonds [82–84]. Although at a higher pH, some cross-reactivity with amino groups can occur, as well as a ring-opening reaction caused by hydrolysis [85], the sulfhydryl specificity and stability of the maleimide group in aqueous solvents can be controlled by the pH of the reaction medium and the choice of maleimide derivative. The selective conjugation of sulfhydryls to maleimides has been applied by linking the thiolated OX26 monoclonal antibodies (MAb) to hydroxypolyethyleneglycol-maleimide [86] and in the attachment of cys-folate to PLL [87] (Table 1d).

3.5. Sulfhydryl (thiol)-reactive cross-linking agents

Another class of heterobifunctional cross-linking agents widely used in conjugation chemistry contain both an amine-reactive group, such as an NHS ester, and a sulfhydryl-reactive end, like the 2-pyridyldithio group in N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) [88,89]. Conjugation with these linkers follows a two-step or multi-step process, offering more control over the route of reaction. The NHS esters are used to form stable amide linkages with primary amines resulting in sulfhydryl-reactive intermediates. In a second step, these intermediates are combined with the sulfhydryl-containing molecule to form a disulfide bond by a thiol-disulfide exchange [90]. These sulfhydryl-reactive intermediates can also be used to create a sulfhydryl group in the molecule to be attached by reducing the disulfide bond with reductive agents like DTT [91]; the resulting free thiol group allows for conjugation with various sulfhydryl-reactive groups, like maleimides or iodoacetalgroups [92]. A sulfhydryl containing RGD-peptide [92] and thiolated transferrin [93] were covalently

bound to PEI and PLL, respectively, using SPDP and DTT (Table 1e).

3.6. Biotin binding to avidin, streptavidin and neutravidin

Avidin and streptavidin consist of four subunits each carrying one biotin binding site in a pocket beneath the protein surface. The multivalent nature of these four binding sites enhances the sensitivity and selectivity for ligand interaction, favoring the use of avidin/streptavidin–biotin systems in immunoassay. Both proteins bind biotin by a non-covalent, biospecific interaction similar to receptor-ligand recognition with a dissociation constant of 1.3×10^{-15} M [94]. Biotin binds to avidin or streptavidin by its bicyclic ring, while the valeric acid side chain is not involved. Therefore, biotinylating agents posses an acylating active group, such as an NHS ester, on the valeric side chain for binding of amine-containing molecules, creating a stable amide bond (Table 1f). NHS-biotin, the simplest biotinylating agent, is insoluble in water, while the sulfo-NHS-biotin can be easily used under aqueous conditions. To enhance the accessibility of biotin to sterically hindered binding sites on streptavidin or avidin, long-chain derivatives, such as N-succinimidyl-6-(biotinamido)hexanoate (NHS-LC-biotin) and sulfo-NHS-LC-biotin, the water soluble derivative, have been developed [95]. To enable the recovery of targeting molecules from biotin binding, derivatives with cleavable long-chains, such as NHS-SS-biotin and sulfo-NHS-SS-biotin, have been introduced [96]. Replication-deficient adenovirus [97], EGF and PLL [98] and diamine polyethylene glycol [44] have been biotinylated using NHS-LC-biotin, NHS-SS-biotin and biotin, respectively, enabling non-covalent interaction with streptavidin, avidin or neutravidin.

4. Biomimetic polymers designed for manufacturing devices exceeding the dimensions of a single cell

In many cases, biomimetic polymers are not designed to interact with individual cells, but rather with multiple cells or even whole tissues. This means that the materials are processed into devices with large surfaces compared to the dimensions of a single cell. Applications include the use as classical biomaterials to replace damaged or lost tissues or as cell carriers in tissue engineering applications. It is obvious that the boundary between these applications cannot be sharply drawn, however, as the signaling from the material surface to cells is an important feature in both cases. In recent years, the field of tissue engineering profited tremendously from the improvement of biomimetic materials as they allow to better control tissue development individual cells. In this approach, many biological aspects ranging from cell attachment to cell differentiation are involved and need to be understood and also controlled. In the following section, we illustrate how biomimetic polymers were designed based on already existing biomaterials.

The polymers used for this approach can be divided into two major classes based on their physicochemical properties hydrogels, water swollen networks composed of hydrophilic polymers [99], from solid lipophilic materials that show little water uptake and at least initially maintain their mechanical properties when brought into an aqueous environment. Both classes exhibit certain advantages with regard to their applications. Hydrogels allow for high diffusion rates of nutrients, drugs and oxygen [100] and can often be injected with or without cells, allowing for a minimally invasive implantation [101]. Furthermore, they can easily adapt to the shape of the defect site by virtue of their flow properties and eventually harden by in situ gelation [101]. The main advantage of rigid polymers is their mechanical stability even after implantation, something that can be achieved for hydrogels only after cross-linking. Furthermore, these materials provide cells with a good environment for processes such as cell adhesion and migration [5].

4.1. Hydrogel materials

Hydrogel polymers for tissue engineering applications range from naturally derived to synthetic materials. Alginate, gelatin, agarose, fibrin, chitosan are examples of naturally derived polymers, whereas poly(ethyleneglycol) (PEG), oligo(poly(ethylene glycol) fumarate) (OPF), poly (acrylic acid) (PAA) derivatives, and poly(vinyl alcohol) (PVA) represent synthetic materials. In the following sections, we will describe a selection of material classes that have been used extensively for the development of biomimetic polymers by the methods described above.

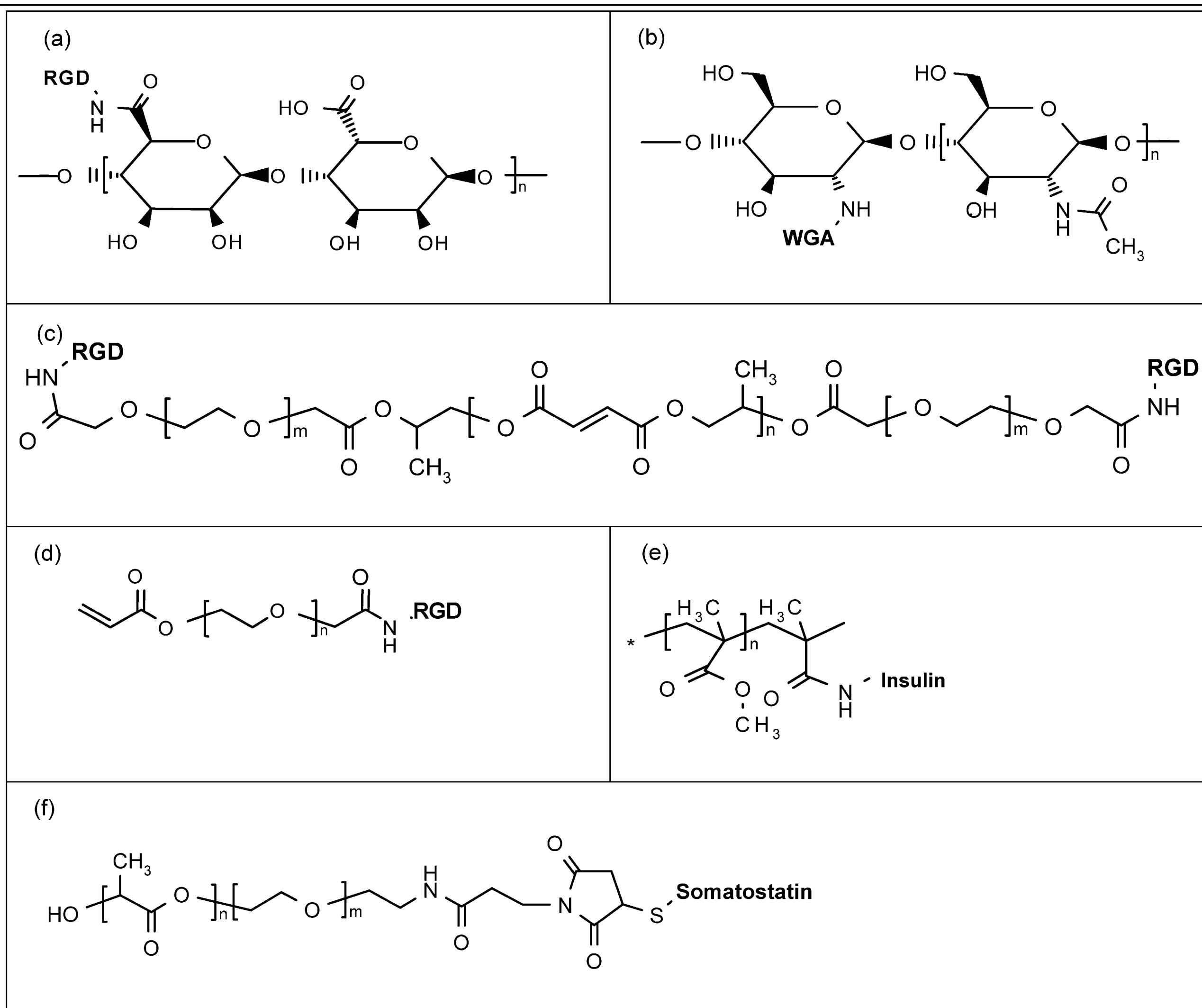
4.1.1. Alginates

Alginate, a linear polysaccharide copolymer of (1–4)-linked β-D-mannuronic acid and α-L-guluronic acid, is widely used due to its low toxicity and ready availability [101]. Its main advantage is its easy modification with peptides due to the many free carboxylic acids on the polymer backbone and mild gelation conditions. Alginate gels can be cross-linked using divalent cations (Ca^{2+}, Ba^{2+}, or Sr^{2+}) or by covalent chemical cross-linking techniques [102,103]. A common approach to improve cell-alginate interactions is to covalently link the integrin binding peptide sequence RGD or its derivatives to the polymer backbone. The free carboxylic groups of the latter are activated using EDC/NHS and reacted with the terminal NH_2-group of the peptide [104–106] (Table 2a). Suzuki et al. tethered a BMP-2-derived oligopeptide to the alginate chains to enhance its suitability in for bone tissue engineering [107].

4.1.2. Chitosans

Chitosan is a linear polysaccharide of (1–4)-linked D-glucosamine and *N*-acetyl-D-glucosamine. It is quite

Table 2
Examples of biomimetic polymers



Materials derived from natural hydrogel type polymers: (a) alginate-RGD; (b) chitosan-WGA. Materials derived from synthetic hydrogel type polymers: (c) PPF–PEG–RGD; (d) PEG–RGD. Materials derived from lipophilic polymers: (e) PMMA-Insulin; (f) PEG–PLA–Somatostatin.

suitable as a substrate for biomimetic polymers not only because of its structure, which is quite similar to the glycosaminoglycans found in native tissue [101], but also because the free amino groups in the polymer backbone are easily modified. Gelation occurs after increasing the pH of the chitosan solution [100,108] or extruding solutions into a non-solvent [108]. The polymer is readily modified by the covalent attachment of molecules with free carboxylic acids using carbodiimide chemistry. Wang et al. covalently bound WGA, a lectin molecule, to chitosan to enhance cell–biomaterial interactions by first activating WGA using EDC and afterwards reacting these products with the amine groups of chitosan to form stable amide linkages [109] (Table 2b).

4.1.3. Fibrin

Another naturally derived polymer is fibrin, a polypeptide. It is naturally formed during blood coagulation from fibrinogen, which is cleaved by thrombin and subsequently covalently cross-linked by factor XIIIa [110,111]. This natural substrate is a suitable candidate for implantation because it is degraded by enzymes. Schense et al. modified this polymer by incorporating the integrin binding adhesion peptides RGD and DGEA [111]. They designed bi-domain peptides, with a factor XIIIa substrate in one domain and a bioactive molecule in the other. During fibrin-cross-linking, the peptides were incorporated in the resulting hydrogel. Zisch et al. used the same method to bind VEGF derivatives to fibrin hydrogels [110].

4.1.4. PEGs

PEGs are very popular synthetic polymers frequently used in tissue engineering and drug delivery applications. Although PEG derivatives provide only endgroups for chemical modification, they are frequently used, because

they are non-toxic and non-adhesive towards proteins, resulting in suitable model systems. In order to process PEG into a hydrogel, each end of the polymer chain must be modified with either acrylates or methacrylates, which are sensitive to photo-cross-linking [101,112]. As an example, PEG with two terminal hydroxyl groups can be converted to an acrylate with acryloyl chloride [112]. To enable sufficient cell–material interactions, like selective cell attachment, RGD-sequences have been grafted to this rather hydrophilic polymer [113–115] (Table 2d). Mann et al. reported reduced ECM production of cells cultured in these hydrogels [13]. They tried to overcome this shortcoming by additionally binding TGF-β1 on a PEG-acrylate-spacer to the polymer via a radical reaction.

4.1.5. Poly(propylene fumarate) derived copolymers with PEG (PEG-PPF)

The amphiphilic triblock copolymer derived from a low molecular weight poly(propylene fumarate) (PPF) with two terminal PEG units represents another class of synthetic hydrogel forming materials and holds great promise for tissue engineering and drug delivery applications. Its aqueous solutions allows for a thermo reversible gelation with final cross-linking of the fumarate double bonds. This results in a system applicable in a minimally invasive manner. Moreover, these PPF-based hydrogels are biodegradable, because they contain several hydrolytically cleavable ester groups in the polymer backbone. Jo et al. synthesized a triblock copolymer consisting of two terminal carboxymethyl PEG units and one PPF block in the middle of the copolymer [116]. The terminal free carboxylic groups allow for conversion to succinimidyl esters using NHS/DCC chemistry resulting in polymers, which could be readily modified with RGD sequences (Table 2c). Numerous other derivatives of fumarate-derived polymers for hydrogel formation have been developed in recent years, such as OPF cross-linked with PEG-diacrylate [117,118].

4.1.6. PAA derivatives

Although acrylic acid derived polymers are known to degrade slowly, they are frequently used as tissue engineering scaffolds due to the easy structure modifications of the resulting hydrogels. In addition, some derivatives such as *N*-isopropylacrylamides show thermoreversible gelation. Stile et al. studied cell–material interactions on *N*-isopropylacrylamide based hydrogels modified with RGD-peptides and heparin-binding FHRRIKA-sequences [119]. Hydrogels were prepared by radical copolymerization of *N*-isopropylacrylamide, acrylic acid and *N*,*N*′-methylenebisacrylamide. The free acid groups stemming from acrylic acid were linked to diamino-PEG using EDC and *N*-hydroxysulfosuccinimide. With sulfosuccinimidyl 4-(maleimidomethyl)-cyclohexane-1-carboxylate, the free amine group of the immobilized PEG was converted to a double bond sensitive to attack from free thiol groups of the peptides. Thus, the integrin-binding RGD-sequences and heparin-binding FHRRIKA-sequences were bound directly to the polymer backbone. A different acrylic acid derivative, *N*-(2-hydroxypropyl)methacrylamide) (HPMA), was altered by tethering RGD sequences or aminosugar residues, which interact with glycosyltransferases on cell surfaces, to the hydrogel [120]. Hydrogels were synthesized by radical copolymerization of HPMA with either RGD or glucosamine derivatives modified with methacryloyl residues.

4.2. Lipophilic and water insoluble polymers

Lipophilic and water insoluble polymers have also been modified to form biomimetic polymers in recent years. Degradable materials are typically chosen for tissue engineering applications, because a gradual resorption of the material is necessary to achieve the ideal complete replacement of the defect with living, functional tissue. Non-degradable materials have, however, been investigated for research applications, to achieve increased biocompatibility or enhanced tissue integration of medical implants. Many non-degradable materials, such as polystyrene (PS) or polyacrylate, have furthermore been modified to yield biomimetic materials. The following chapter represents a selection of materials, more of which are described in the literature [121–124].

4.2.1. Polystyrene

Although it is not biodegradable, PS provides a good model system for lipophilic surfaces. To make use of the cell culture approved polymer, Park et al. synthesized a sugar-bearing PS derivative with RGD grafted to the polymer backbone using carbodiimide chemistry to investigate the changes in the behavior of hepatocytes on these modified polymer surfaces [125]. Ito also linked insulin to non-degradable PAA chains and grafted them to standard PS films [126].

4.2.2. Poly(methylmethacrylate) (PMMA)

PMMA can easily be modified following the hydrolysis of some methyl ester groups in a basic environment. Peptide sequences can be bound to PMMA surfaces through subsequent reaction of the obtained acid residues with amine groups of peptides using EDC chemistry [127] (Table 2e). An alternative constitutes the tethering of the bioactive molecule to an acrylate anchor and grafting this molecule to the PMMA backbone using UV-irradiation. Schaffner et al., for example, used this method to covalently link insulin to PMMA surfaces [128].

4.2.3. Poly(lactic-co-glycolic acid) and poly(lactic acid)

The most frequently used materials for tissue engineering applications are poly(lactic-*co*-glycolic acid) (PLGA) [129] and poly(lactic acid) (PLA) [130], because of their excellent biocompatibility, their FDA approval, and the established procedures to form rigid scaffolds for the cultivation of cells.

PLGA chains are terminated with a free carboxyl group, which can be used for modification of the polymer. In one example of PLGA modification, a galactose derivative was bound directly or via a PEG spacer to the acidic end of the molecule [129]. Using NHS/DCC chemistry, an amine containing galactose derivative or PEG diamine was tethered to the polymer. Lactobionic acid was then grafted to the remaining free amine group of PEG using carbodiimide chemistry. As the regular PLA chain contains few reactive centers and is also prone to hydrolysis, an alternating block copolymer of lactic acid and lysine was used to provide free reactive amine groups in the polymer backbone [131]. RGD peptides were then covalently attached to the resulting free amine groups using CDI as connecting molecule.

A new class of active PLA derivatives was designed by Tessmar et al. [25] (Table 2f). To reduce uncontrolled protein adsorption to the lipophilic PLA, a diblock copolymer with hydrophilic PEG was synthesized, starting from PEG and D,L-lactide in the presence of stannous 2-ethylhexanoate [132,133]. The PEG chain terminates with an amine group presenting a possible modification site. To activate this polymer for protein attachment, the amine group was converted to a reactive carboxylic group using L-tartaric acid or succinic acid as a linker with standard carbodiimide chemistry. Alternatively, a thiolreactive group was introduced via β-alanin and maleic acid anhydride resulting in a thiol reactive maleinimide. Insulin, as an aminecontaining protein, and somatostatin, a substance with a cleavable disulfide bridge, were shown to attach to these activated polymers. To process the active polymers, Hacker et al. developed a new anhydrous method for scaffold fabrication to maintain the binding activity, resulting in highly porous cell carriers, which can be easily modified with proteins for use in tissue engineering [24].

5. Biomimetic polymers designed for manufacturing devices with sizes below the dimensions of a single cell

In this chapter, we will shed some light on biomimetic polymers developed especially for the manufacture of nanoparticulate delivery systems. Biomimetic nanoparticles hold great promise to facilitate the cellular uptake of drugs and DNA as well as for drug targeting applications. In contrast to many of the materials used for the interactions with tissues and multiple cells, a prominent design feature of the materials described here is that many of them are amphiphilic or have a block copolymer structure that facilitates the manufacture of colloidal aggregates.

Unfortunately, following intravenous administration, most particulates are rapidly removed from the bloodstream by the reticuloendothelial system (RES), typically due to phagocytosis by macrophages [134] in the liver and spleen, limiting the efficiency as drug delivery system. The formation of nanoparticles with an outer hydrophilic shield consisting of PEG [135], poloxamer [136], albumin [137], cyclodextrine [138,139] or transferrin [140] reduces unspecific cell adhesion, minimizing the rapid clearance by the RES, providing long circulating drug delivery systems [141,142].

EGF-antibodies, such as B4G7, growth factors like EGF or FGF, transferrin, or vitamins like folic acid or biotin were applied as targeting agents, because of the well-known over-expression of the corresponding cell surface receptors on tumor cells [143–145].

Again, a plethora of materials have been developed in recent years, which we cannot review exhaustively, but rather only on the basis of selected examples. We will thereby distinguish between materials that have primarily been designed for the delivery of drugs and those that have been designed for the delivery of DNA, which also have to condensate the DNA with the help of cationic building blocks.

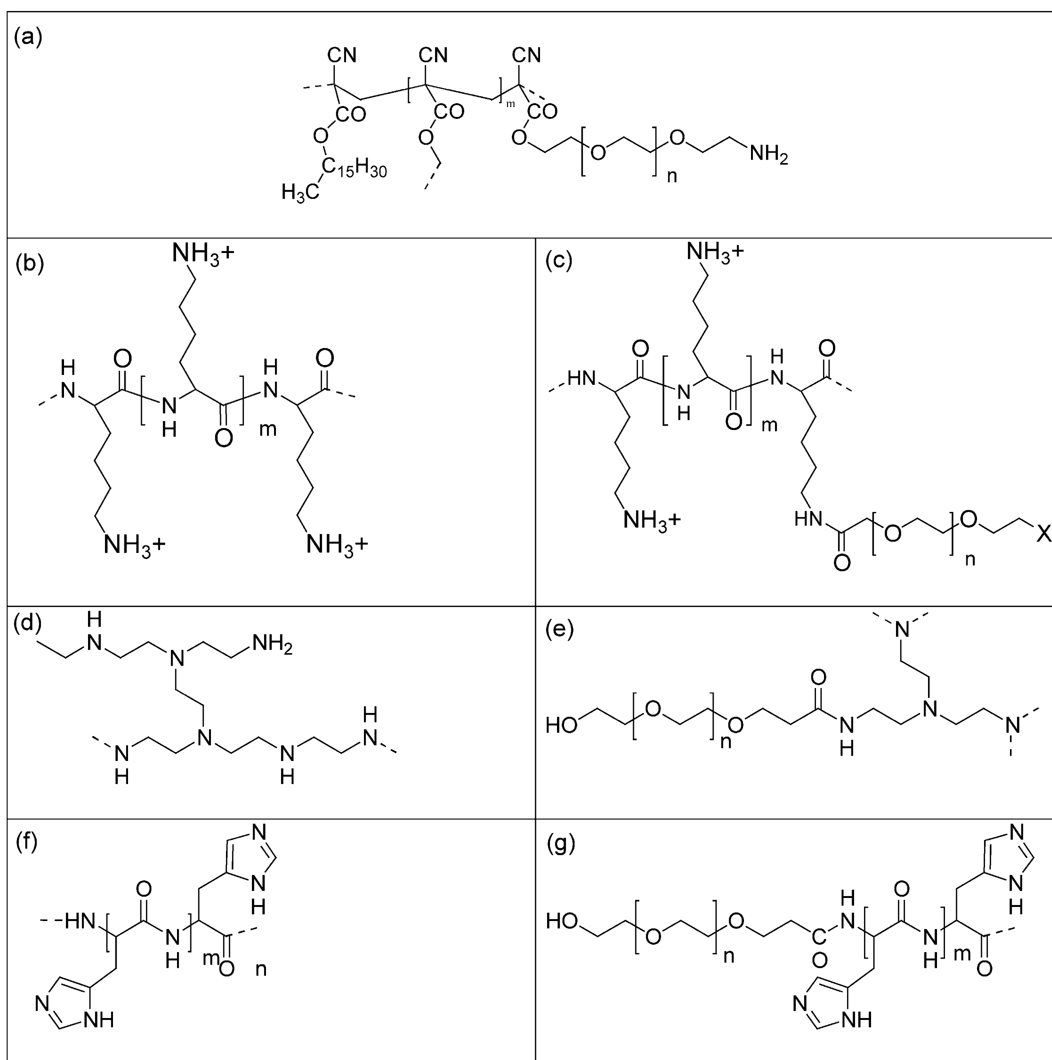
5.1. Polymers for the preparation of nanoparticles for drug delivery

5.1.1. Polyacrylate-blockcopolymers

Stella et al. synthesized a poly(aminopoly(ethylene glycol)cyanoacrylate-*co*-hexadecyl cyanoacrylate) (poly (H_2NPEGCA-*co*-HDCA)) copolymer involving derivatization of the monomers followed by polymerization. The biodegradability of the copolymer was introduced by connecting the *N*-protected aminopoly(ethylene glycol) or *n*-hexadecanol to the cyanoacrylate backbone. The PEG-coated nanoparticles were prepared by subsequent precipitation. In contrast to other coupling strategies, which involve several reactive groups accessible for conjugation, the NHS ester of folic acid was selectively attached to the terminal amino group of the hydrophilic PEG block of the preformed poly(H_2NPEGCA-*co*-HDCA) nanoparticles [72] (Table 3a). Li et al. encapsulated DNA into poly(H_2NPEGCA-*co*-HDCA) nanoparticles using a water–oil–water solvent evaporation technique and coupled transferrin selectively to the terminal amino group of the PEG chains by reductive amination, establishing a potential delivery system of therapeutic genes to the target cells [146].

Pan et al. followed an elegant strategy leading to small shell cross-linked nanoparticles with an amphiphilic core–shell morphology, a rather unique design for a biocompatible long-circulating drug carrier system. The diblock copolymer poly(acrylic acid)-*b*-polyisoprene (PAA-*b*-PI) was synthesized by nitroxide-mediated radical polymerization of *tert*-butyl acrylate and isoprene. Micelles were further stabalized by the intramicellar cross-linking of acrylic acid residues located within the shell domain of PAA-*b*-PI nanoparticles, using a homobifunctional diamino-cross-linking agent [147]. The remaining free carboxylate groups were activated with a water-soluble carbodiimide and coupled selectively with the terminal amino group of a folate tagged PEG-amine (Fig. 2).

Table 3
A few examples of polymers used for the preparation of nano-scaled materials



(a) Poly(H_2NPEGCA-*co*-HDCA) synthesized by Stella et al. [72]. (b) PLL. (c) PLL–PEG, synthesized by Leamon et al. [79]. (d) PEI. (e) PEI–PEG, synthesized by Ogris et al. [136]. (f) Poly(HIS). (g) Poly(HIS-PEG), synthesized by Lee et al. [73].

5.1.2. Poly(ethylene glycol)-co-poly(caprolactone) (PEG-PCL)

Gref et al. prepared a unique model system for the study of cell–material interactions enabling tagging with any biotinylated ligand or even multiple ligand binding on the surface of engineered nanoparticles. For the synthesis of the amphiphilic PEG–PCL diblock copolymer, poly(ethylene glycol)-bis amine was conjugated to biotin by carbodiimidazole-mediated amide synthesis directed primarily to obtain the mono-biotinylated amino-PEG derivative [44]. The remaining amine group has been used as the initiator for the polymerization of ε-caprolactone, catalyzed by stannous octanoate, to give the biotinylated PEG–PCL-copolymer. From this polymer, nanoparticles were formed by nanoprecipitation, in part using mixtures of biotin-PEG–PCL and PEG-PLA. The nanoparticle suspension can be incubated in avidin solutions and the final particles isolated by centrifugation. As a model substance, a biotinylated lectin, WGA, has been attached to the nanoparticle surface by adding it to the avidin-coated nanoparticle suspension. The potential use of these nanoparticles as drug delivery systems for oral or even intravascular administration, as proposed by Gref et al., must still be investigated.

5.1.3. Poly(ethylene glycol)-co-poly(L-lactic acid)

Olivier et al. described the so-called immunonanoparticles consisting of a mixture of methoxypoly(ethylene glycol)-*co*-poly(L-lactic acid) (methoxy-PEG-PLA) and maleimide-poly(ethylene glycol)-*co*-poly(L-lactic acid) (maleimide-PEG-PLA)) tagged with MAb to the rat transferrin receptor [86]. The MAbs undergo receptor-mediated transcytosis across the brain microvascular barrier

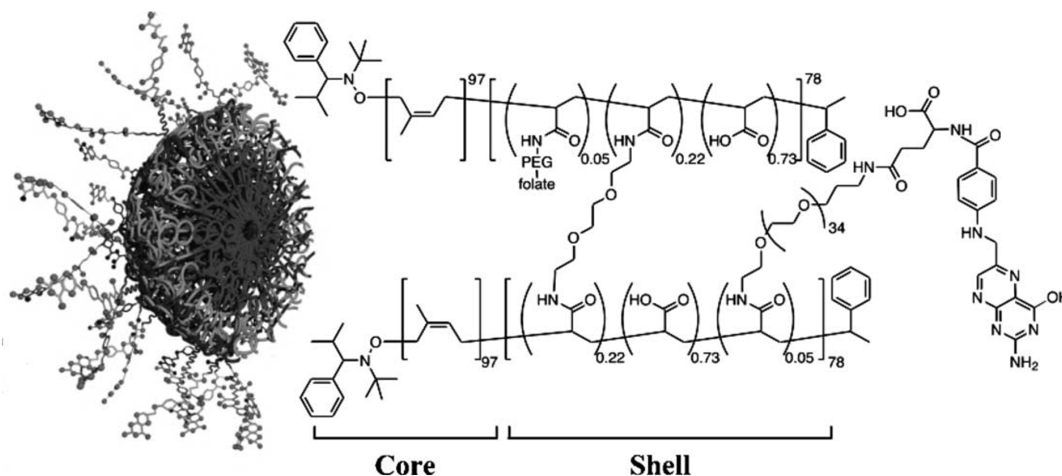


Fig. 2. Intramicellar cross-linked [poly(acrylic acid)-*b*-polyisoprene]nanoparticles. A part of acrylic acid residues of PAA-*b*-PI micelles were activated followed by conjugation with a diamino linker to achieve intramicellar shell cross-linking. The remaining acrylic acid groups were coupled with the folate-PEG amine to prepare folic acid-conjugated shell cross-linked nanoparticles. Reproduced from Pan et al. [147] by permission of The Royal Society of Chemistry.

via the endogenous blood–brain barrier transferrin transport system, enabling drug delivery targeted specifically at the brain. The copolymers were synthesized by ring-opening polymerization of L-lactide on the terminal hydroxyl group of the corresponding methoxy-poly(ethylene glycol) or maleimide-poly(ethylene glycol), catalyzed by stannous octanoate. The desired targeting peptide had been thiolated on the primary amino group using Traut's reagent. The nanoparticles were prepared using an emulsion/solvent evaporation technique using blends of methoxy-PEG-PLA and maleimide-PEG-PLA. The targeting peptide was then conjugated by the formation of a stable thioether to the PEG-shield of the prefabricated nanoparticles.

5.1.4. Poly(L-histidine)-co-poly(ethylene glycol) (Poly(His)-PEG): poly(ethylene glycol)-co-poly(L-lactic acid) -mixtures

Poly(His)-PEG is a copolymer that forms nanoparticles containing a pH-sensitive [148] biodegradable and fusiogenic [149] poly(L-histidine) (poly(His)) inner core, shielded by an outer PEG layer. Poly(His) has been synthesized by a base-initiated ring-opening polymerization of protected *N*-carboxy anhydride (NCA) of L-histidine and has been coupled to carboxylated PEG [73]. To achieve a selective internalization of the nanoparticles by tumor cells, DCC and DMAP-mediated ester formation has been used to covalently bind folic acid and aminated folic acid to the terminal hydroxyl group of the PEG blocks of the N-protected poly(His)-PEG-copolymer and PEG–PLA, respectively. The nanoparticles were prepared using blends of different weight ratios of PEG–PLA and poly(His)-PEG to control the pH-sensitivity and stability of the micelles. The nanoparticles were loaded with the anti-tumor drug adriamycin (ADR), purified by dialysis and isolated by lyophilization (Tables 2f, 3f and g).

5.2. Polymers for non-viral gene delivery

Polycations spontaneously condense DNA due to the strong ionic interaction with the negatively charge phosphorous groups of the DNA backbone, leading to the formation of nanometer-sized particles, known as polyplexes [150].

The efficacy of the DNA complexation depends on the molecular weight and cationic charge density of the polymer and is important for the protection of DNA in vitro and in vivo and also for the stability of the resulting complexes [151]. Since most of these complexes enter the cells via unspecific endocytosis [152,153], the conjugation of a hydrophilic shield on the surface of the polyplexes reduces the competing unspecific cell adhesion in favor of the specific receptor-mediated uptake enabled by attached targeting molecules.

It has been shown that the ligand coupling using long PEG spacers improves the accessibility for receptor binding, leading to better cellular uptake and to reduced cytotoxic side effects [87,150].

Unfortunately, the direct PEGylation of the cationic polymers (pre-PEGylation) leads to derivatives with reduced DNA complexation efficacy. To overcome this problem, methods have been established to conjugate PEG to the pre-formed polyplexes (post-PEGylation) [154,155].

Below we will describe a few materials that have been used for DNA delivery that have been modified to achieve better efficiency with biomimetic principles. Many of them are derived from polycationic polymers, which were altered by the formation of block copolymers and/or the attachment of biologically active entities to allow for better cellular uptake and also extended bioactivity.

5.2.1. Poly(L-lysine) derivatives

PLL itself has been widely used as non-viral vector for gene delivery, favored due to the biodegradability of

the polypeptide and accessibility within a broad molecular weight range.

The ε-amine groups in the side chain of the polyamide backbone exhibit multiple cationic charges in an aqueous environment at physiological pH. Several targeting molecules, such as growth factors, vitamins, transferrin and carbohydrates, have been tagged to PLL by conjugation to the primary ε-amine groups (Table 3b). Unfortunately, the majority of the delivered PLL–DNA polyplexes remains sequestered within the endosomal–lysosomal compartment, which dramatically reduces transfection efficiency [156,157].

Different research groups have supplemented polyplexes with endosomolytical substances, such as adenovirus [71,79,97,158,159], chloroquine [144,160], or endosome disruptive peptides [161,162], facilitating the release of the polyplexes from the endosome, yielding improved gene expression. Merwin et al. conjugated the T101 antibody, which specifically binds to the CD5 moiety exhibited on T lymphocytes, to PLL using carbodiimide chemistry. The specificity and relative amount of interaction of the corresponding polyplexes with cells expressing the CD5 moiety was observed using the iodinated T101 derivative [71].

B4G7, a mouse monoclonal antibody, which is uniquely internalized by EGF receptor-mediated endocytosis, has been tagged to PLL through a stable disulfide bond by disulfide exchange with PLL-SH and B4G7-SS-pyridine using SPDP and DTT [163]. The extent of antibody-binding was evaluated by the binding assay using [^{125}I] B4G7 and a competitive inhibition assay.

To achieve tumor cell targeting, the NHS ester of folic acid has been covalently bound to PLL by acylation of the primary amine functions of the polymer [157]. Transferrin, a carbohydrate residue containing protein, has been tagged to the polymer by sodium periodate oxidation and subsequently reductive amination [79,158] or also by disulfide linkage [93]. The corresponding polyplexes were formed after the conjugation of the targeting molecule.

Asialofetuin, a natural ligand of the hepatocyte-specific ASGPr and the artifical ligand tetragalactose-peptide, have been coupled to PLL via disulfide linkages [164]. The tetragalactose has been linked to a synthetic peptide by reductive amination using sodium cyanoborohydride and subsequent coupling to PLL. Both vectors were used in transfection experiments evaluating their targeting properties in direct comparison. A similar approach has been taken by Erbacher et al., who link galactose and lactose to PLL using isothiocyanate as a linker to prepare liver targeted non-viral vectors [81].

5.2.2. PLL–PEG-copolymers

To increase the mobility of the used targeting molecule, hydrophilic PEG can also be used as a spacer with the cationic PLL (Table 3c). In another attempt to target the folate receptor, folate-γ-cysteine was covalently bound to *N*-(hydroxysuccinimidyl-poly(ethylene glycol)-maleimide (NHS-PEG-maleimide) at the maleimide end of the polymer [87]. Then prefabricated PLL–DNA polyplexes were mixed with the folate-PEG-NHS and a folate-tagged PEG shield was covalently bound to the polyplex surface by the newly formed amide linkage.

5.2.3. Non-covalent conjugates of PLL

Another approach to actively targeting PLL takes advantage of the non-covalent attachment of targeting molecules using the ionic biotin–avidin/streptavidin-interaction. This conjugation strategy enables the attachment of any biotinylated or streptavidinylated targeting molecule to the corresponding match, creating a 'universal' vector for a variety of different targeting sites. Here, transfection experiments were performed to clarify the influence of complex structure on transfection efficiency in vitro, while the ability of in vivo applications still remains untested. Xu et al. attached EGF to PLL of varying chain lengths by biotinylating both EGF and PLL using NHS-SS-biotin [98]. The conjugation was then initiated by the addition of avidin, streptavidin or neutravidin followed by DNA complexation, using mediums with low and high ion concentration.

Wagner et al. conjugated replication-deficient adenovirus both covalently and non-covalently to PLL to assure the colocalization of the endosomolytically active adenovirus and the PLL–DNA polyplexes in the endosomal–lysosomal compartment. The covalent linkage was facilitated by a transglutaminase reaction [97]. To enable the non-covalent attachment, streptavidin has been conjugated to mercaptopropionate-linked PLL by a stable disulfide bond using SPDP-modified streptavidin. Adenovirus has been biotinylated using NHS-LC-biotin, facilitating the optimal accessibility of biotin for the four binding sites of streptavidin. DNA was added to the corresponding adenovirus-PLL conjugates to form the so-called binary complexes, leading to a non-viral vector combining both DNA complexation and endosomolysis. To achieve active tumor targeting, transferrin-tagged PLL chains, formed via reductive amination, were added to the binary complexes, leading to the so-called ternary complexes.

5.2.4. Polyethylenimine derivatives

Because of the chemical structure of the trivalent amine, PEI exists in two forms, as either a linear or branched polyamine (Table 3d). By combining a high transfection efficiency and endosomolytical properties, enabling the accelerated release of PEI–DNA-polyplexes from the endosomal–lysosomal compartment, PEI prevails as a promising polymer for the design of non-viral vectors [152,165].

Several different targeting molecules have been tagged to polyamines to achieve active and specific transport of the DNA-polymer polyplexes into the cell interior. To achieve ASGPr-mediated polyplex uptake, galactose-bearing PEI has been prepared by reductive amination and was then used for DNA complexation [78]. Similar to this approach,

Bettinger et al. conjugated tetragalactose to PEI, confirming receptor selectivity by direct comparison to the tetraglucosylated PEI derivative [166].

Moreover, RGD peptides were also covalently bound to PEI to achieve specific cell adhesion, enhancing the cellular uptake [92]. Here, sulfhydryl-terminated RGD-peptides were used, facilitating the covalent attachment by disulfide bonds, formed by a SPDP-mediated disulfide exchange.

5.2.5. *Poly(ethylene glycol)-co-poly(ethyleneimine)*

Using hydrophilic diblock copolymers (Table 3e), a transferrin-tagged poly(ethylene glycol)-*co*-poly(ethyleneimine) (PEG-PEI) has been synthesized by coupling transferrin to PEI using sodium periodate oxidation and reductive amination with sodium cyanoborohydride [135, 167]. The polyplexes were formed with plasmid DNA and PEGylated by adding the commercially available NHS ester of propionic acid PEG to the polyplex suspension (post-PEGylation). In both cases, improved transfection efficiency has been observed in in vitro and in vivo experiments, which has been attributed to the effective shielding properties of both PEG and transferrin as well enhanced cell uptake, due to the specific targeting by transferrin conjugation.

5.2.6. *Non-covalent conjugates of PEI*

Similarly to PLL, EGF was also non-covalently bound to PEI. The NHS ester of biotin–PEG was thereby linked to EGF via an amide bond leading to mono-and multi-PEGylated EGF derivatives. Afterwards, streptavidin was attached to the PEI–DNA polyplexes by ionic interaction and then mixed with the EGF-tagged biotin–PEG, leading to non-covalently bound complexes joined by the biotin–streptavidin interaction [168].

6. Examples for applications in tissue engineering

Biomimetic materials, in general, hold a great potential for specifically controlling cellular functions and behavior, which is of tremendous importance, where the creation of new tissues is concerned. Here, we will illustrate that by giving a few examples from the field of tissue engineering.

To demonstrate the retained bioactivity of peptide sequences tethered to fibrin hydrogels, Schense et al. investigated the neurite outgrowth in hydrogels modified with the adhesion-mediating sequences RGD or DGEA, which exhibit different integrin specificity, or the non-adhesive sequence RDG [111]. Dorsal root ganglia from 8-day-old white chicken embryos were individually embedded in the different three-dimensional hydrogels. Additionally, soluble peptides were added to the hydrogel as a control, serving as competitive inhibitors. After 48 h of culture, the incorporation of RGD resulted in reduced neurite outgrowth, whereas DGEA enhanced neurite outgrowth as expected. The use of RDG or supplemented soluble peptides led to the same level of neurite outgrowth as in unmodified fibrin (Fig. 3).

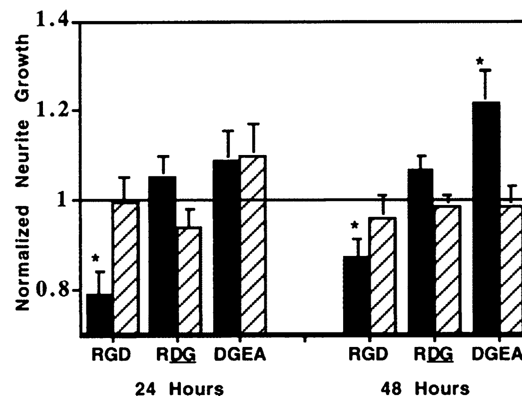


Fig. 3. The effect of tethered peptide on neurite outgrowth in fibrin gels. All tested hydrogels were modified with covalently linked peptides. Cells were cultured with (hatched bars) or without (solid bars) soluble peptides additonally supplemented to the culture medium. (*) means $P < 0.05$ compared to the unmodified hydrogel. Error bars indicate standard deviation from the mean ($n = 3$). Reprinted with permission from Schense et al. [111]. Copyright (1999) American Chemical Society.

Other hydrogels, like OPF derived hydrogels, were also modified with RGD sequences to promote the specific binding of marrow stromal cells [118]. Shin et al. investigated the influence of the polymer PEG chain length, cross-linking density, and the preincubation of MSCs with soluble RGD peptides on the extent of cell adhesion. Longer PEG chains, attached as peptide tethers, and previous blocking of the integrin receptors on the cell surfaces led to reduced cell adhesion, whereas the cross-linking density had no effect on cell behavior. These results suggest that MSC attachment on the previously non-adhesive OPF gels can be achieved by means of peptide incorporation and an appropriate length of the peptide anchorage chain (Fig. 4).

The effect of various sugar-modified PLGA and PEG–PLGA diblock copolymers on hepatocyte cell attachment was examined by Yoon et al. [129]. Hepatocytes were isolated from 40-week-old male Sprague–Dawley rats and their attachment on different surfaces was investigated. The results indicate that galactose enhances cell attachment better than glucose, with a maximum blend ratio of 1% of sugar-modified to unmodified polymer. Introduction of PEG spacers decreased the overall amount of attached cells; longer PEG chains resulted in even fewer adsorbed cells.

Besides the attachment of adhesion-mediating peptides, there are also applications where bigger proteins, like growth factors, are attached to the polymer surface, leading to extended bioactivity and distinct localization of the factor.

Suzuki et al. investigated the in vivo effect of BMP-2 derived oligopeptides on ectopic bone formation [107]. The peptides were either covalently linked or physically mixed into an alginate gel and 10 mg of the gel were injected in the calf muscle of Wistar rats. After 3 and 8 weeks, the implanted region was removed and stained

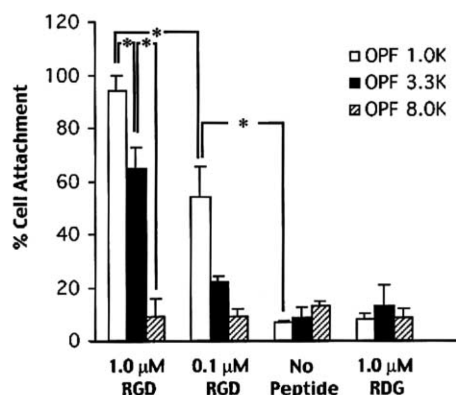


Fig. 4. Percent cell attachment on different OPF hydrogels. rMSCs seeded on hydrogels fabricated by crosslinking OPF with PEG diacrylate. 1.0, 3.3, 8.0 K represent the number average molecular weight of PEG prior to OPF synthesis. H 1X, H 3X, and H 5X, indicate a 1:1, 3:1, and 5:1 ratio of double bonds in PEG-diacrylate to those in OPF, respectively, correlating to cross-linking density of the resulting hydrogels. Error bars indicate standard deviation from the mean ($n = 3$). Reproduced from Modulation of marrow stromal osteoblast adhesion on biomimetic oligo[poly(ethylene glycol) fumarate] hydrogels modified with Arg-Gly-Asp peptides and a poly (ethyleneglycol) spacer, Shin et al., Copyright© Wiley Periodicals, Inc., 2002 [118]. Reprinted by permission of John Wiley Sons, Inc.

with hematoxylin and eosin or van Kossa stain followed by microscopic observation. Implant groups with covalently linked oligopeptides showed osteoblast ingrowth and mineralization in the pores of alginate hydrogels after 3 weeks (Fig. 5A) and abundant trabecular bone formation was reported after 8 weeks of implantation. On the other hand, the control group with the non-covalently bound BMP-2 derivative showed no mineralization after 3 weeks (Fig. 5B) and after 8 weeks the hydrogel was completely bioabsorbed.

The effect of immobilized insulin on the culture of Chinese hamster ovary (CHO) cells was studied by Ito et al. [127]. Insulin grafted on PMMA films enhanced the proliferation of CHO not only compared to unmodified PMMA films, but also with regards to the addition of the same amount of free insulin. After harvesting the cells by EDTA treatment, new cells could be cultured on the films. Up to four utilizations were performed with only a slight decrease in insulin activity, possibly due to coverage of the films with proteins secreted from the growing cells.

7. Applications of nano-scaled materials

Under aqueous conditions, amphiphilic copolymers self-assemble into micelles containing a hydrophobic core surrounded by a shell composed of the hydrophilic blocks [169]. Different methods, such as diafiltration, dialysis, nanoprecipitation or emulsion techniques, have been used for the preparation of nanoparticles, which have been widely used as nanocontainers for drug and plasmid DNA delivery or in immuno assays [170].

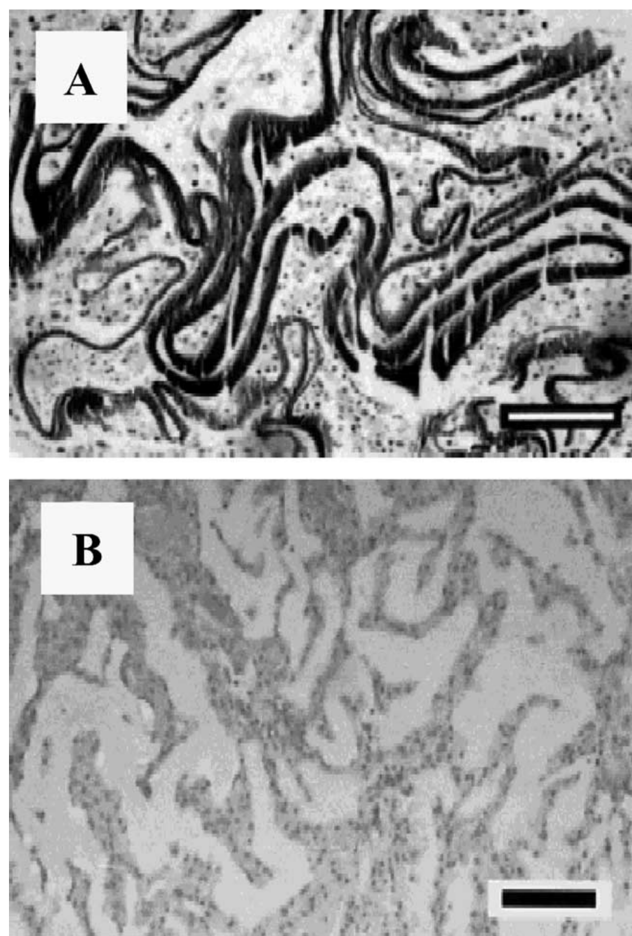


Fig. 5. Photomicrographs of alginate hydrogel implants modified with a BMP-2 derivative. Von Kossa staining after three weeks of implantation in calf muscle of rats. Scale bar 100 μm. (A) Implants with covalently linked peptide. Black stains indicate mineralization. (B) Implants with mixed peptide show no mineralization. Reproduced with slight modifications from Alginate hydrogel linked with synthetic oligopeptide derived from BMP-2 allows ectopic osteoinduction in vivo, Suzuki et al., Copyright© John Wiley and Sons, Inc., 2000 [107]. Reprinted by permission of John Wiley Sons, Inc.

A variety of biocompatible and biodegradable polymers have been used for the preparation of nanoparticles using folic acid as a tumor targeting unit, among them poly(H_2-NPEGCA-*co*-HDCA) and PEG–PLA or PEG-His-copolymers. Poly(H_2NPEGCA-*co*-HDCA) nanoparticles were tagged with folic acid to an extent of 14–16% calculated on the total number of PEG chains (Fig. 6) [72]. The recognition efficacy of the attached folic acid by the folate binding protein (FBP), the soluble form of the folate receptor, was demonstrated by surface plasmon resonance analysis, enabling the real-time analysis of the molecular association. FBP was immobilized on an activated dextran-coated gold film on the surface of a sensor and the folic acid-tagged nanoparticles were allowed to interact with the modified surface of the sensor, revealing even lower dissociation constants compared to free folic acid. Stella et al. attributes the greater binding affinity of

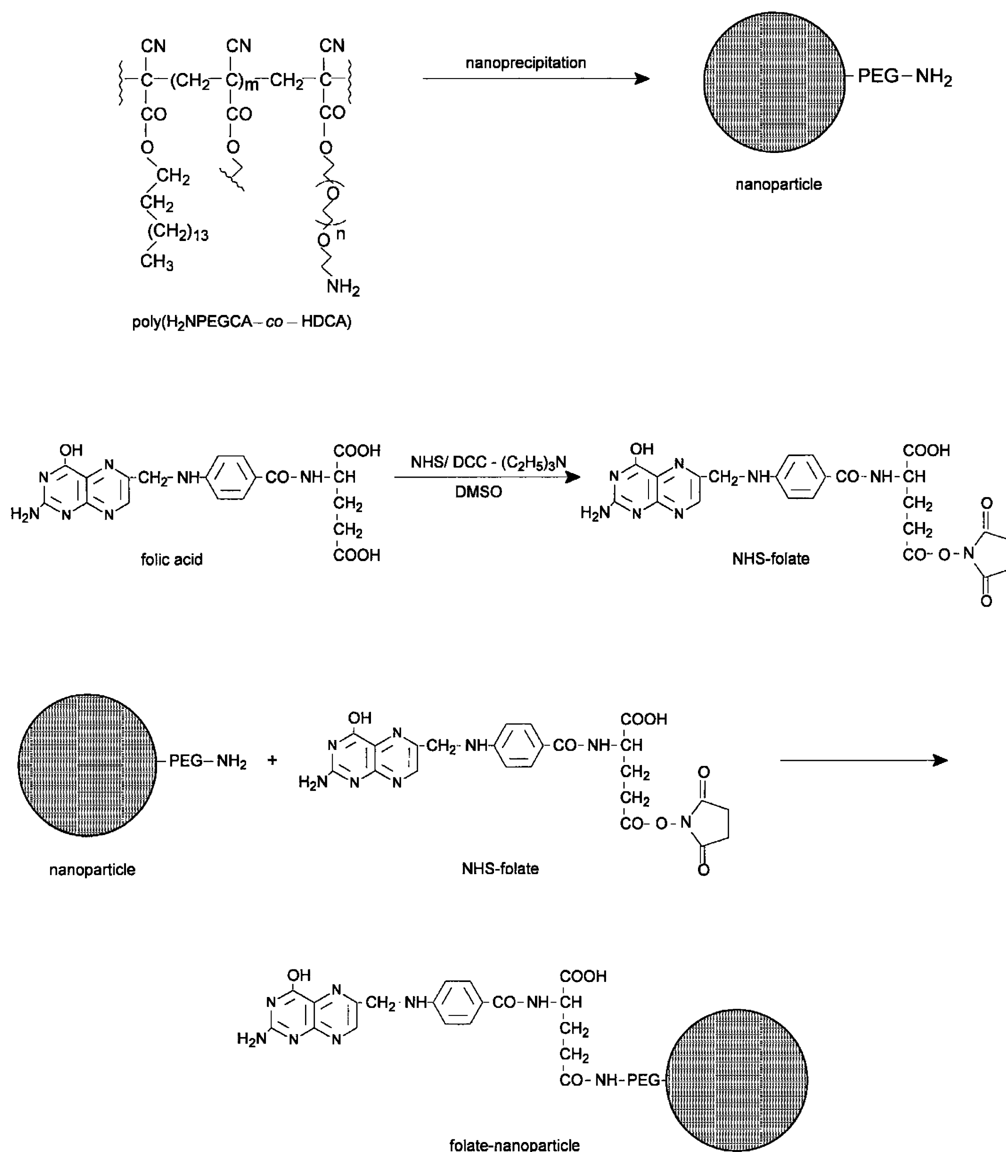


Fig. 6. Preparation of poly(H_2NPEGCA-*co*-HDCA) nanoparticles and conjugation with folic acid. Nanoparticles with an outer amino-PEG layer were prepared by nanoprecipitation of poly(H_2NPEGCA-*co*-HDCA). In a second step folic acid was transformed to the succinimidyl ester, using DCC, NHS, and conjugated to the terminal amino group of the PEG block on the nanoparticle surface. Reproduced from design of folic acid-conjugated nanoparticles for drug targeting, Stella et al., Copyright© Wiley-Liss, Inc. and the American Pharmaceutical Association 2000 [72]. Reprinted by permission of John Wiley Sons, Inc.

the folate-conjugated nanoparticles to the stronger interaction with the FBP receptor clusters with the multivalent form of the ligand folic acid on the nanoparticle surface. The corresponding nanoparticles lacking the folic acid tag, did not associate with the immobilized FBP.

Lee et al. conjugated folic acid to the PEG shield of pH-sensitive poly(His-PEG) and PEG–PLA blended poly (His-PEG) nanoparticles, incorporating ADR [73]. The application of a mixture of polymers for the preparation of nanoparticles increased their stability against dissociation and facilitated the controlled pH-dependent release of the antitumor agent triggered by only slight changes in the pH, similar to those measured in the tumor interstitial fluid. The cytotoxic effect of ADR was evaluated using folic acid-tagged nanoparticles as well as non-targeted nanoparticles with human breast adenocarcinoma cells, confirming that the cytotoxicity of ADR-loaded nanoparticles was dependent on the pH of the environment. The conjugation with folic acid increased the cytotoxicity, indicating an enhanced uptake of nanoparticles by endocytosis. This effect could even be augmented by the fusiogenic effect of poly(His), facilitating the endosomal release of ADR after the particle uptake by human breast adenocarcinoma cell (MCF-7).

Another approach of active targeting has been followed by Li et al.: the coupling of transferrin, an iron-transporting serum glycoprotein, onto the surface of PEG-coated biodegradable polycyanoacrylate nanoparticles to deliver incorporated plasmid DNA as a therapeutic device into tumor cells [146]. The DNA was microencapsulated utilizing a double emulsion technique with the addition of

polyvinyl alcohol to prevent the relaxation of DNA into the linear form, which exhibits less efficient gene expression [171]. The cell association studies were performed with K562 cells, using tagged and untagged nanoparticles, revealing an improved target cell binding. The application of free transferrin decreased the extent of association of the transferrin-labeled nanoparticles with the cell surface, confirming the selectivity of the receptor interaction.

Gref et al. prepared nanoparticles from biotinylated PEG–PCL-copolymer enabling the attachment of any ligand, or even a multiple ligand coupling, by taking advantage of the biospecific interaction of biotin and avidin [44]. The PCL-block displays the hydrophobic core, which can be use for drug incorporation, while the flexible PEG blocks serve as spacer for the biotin coupling, enabling maximal accessibility for the biotin-binding site beneath the avidin surface. The nanoparticles were prepared using biotinylated PEG–PCL and PEG–PLA blends and were obtained in a size range of 90–100 nm, which only slightly increased after the binding of avidin. Biotinylated WGA, a model lectin, which specifically recognizes cell surface carbohydrates, such as *N*-acetyl-D-glucosamine and *N*-acetylneuraminic acid, was used to target anticancer drugs to colon carcinoma cells. Nanoparticles consisting of PLA, PEG–PLA, PEG–PCL and ligand-decorated PEG–PCL were used in cell association and cytotoxicity experiments performed on the human colon adenocarcinoma cell line Caco-2, measuring the cell-associated radioactivity by incorporating radioactively labeled PLA into the core of the nanoparticles. Only the WGA-tagged nanoparticles showed specific interaction with the cell surface, leading to a 12-fold increase in cell association. The biotin labeling enables the attachment of any biotinylated ligand by the addition of avidin, facilitating a broad use in the design of drug delivery systems (Fig. 7).

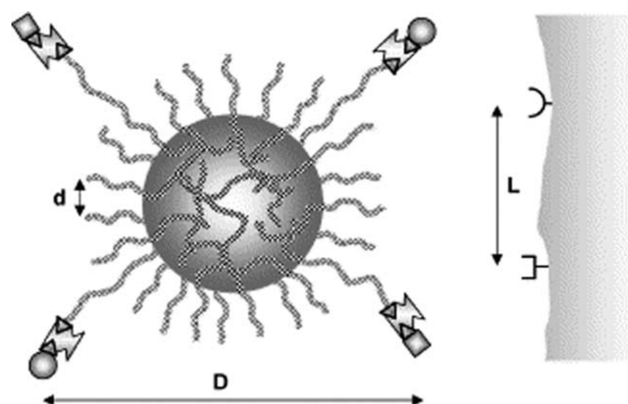


Fig. 7. Schematic representation of a core-corona nanoparticle coated with a PEG 'brush' (distance d between two terminally attached PEG chains). Several PEG chains carry a covalently linked biotin molecule (△), which binds one avidin molecule (⌘). Three biotin binding sites remain available to enable the further attachment of different biotinylated ligands, separated by a distance D, through interaction with avidin. The functionalized nanoparticle (left) could further interact with a target cell (right) bearing two different surface receptors at a mean distance L from one another. Reproduced from Gref et al. [44].

8. Applications for non-viral gene delivery

Gene therapy could become a promising tool for the treatment of inheritable or acquired diseases by delivering DNA into living cells to correct genetic abnormalities [172].

Viral vectors provide high transfection efficiency and can deliver DNA into specific cell populations. The risk of immunogenic or toxic reactions triggered by the viral components of the vector, however, as well as viral recombination or undesired activation of potential oncogenic sequences, restricts their application in human gene therapy [173–175]. Despite a lower transfection efficiency and limited duration of the resulting gene expression, using non-viral vectors for this purpose may be a promising strategy to overcome such difficulties [176]. Unfortunately, most non-viral vectors provoke membranolysis or host cell complexation, leading to a tremendous loss of viable transfected cells. To enhance the transfection efficiency in specific cells and reduce cytotoxic effects on other tissues, targeting molecules have been attached.

To achieve better cell specifity, Merwin et al. conjugated T101 murine MAb, which bind to the CD5 moiety on the surface of T lymphocytes, covalently to PLL [71]. Jukat cells and T lymphocytes, as CD5 positive cells, were used in a radioactive competitive cell binding assay. To examine the specificity of receptor binding, the HUH-7 hepatocyte cell line without the CD5 moiety was used as a negative control. Sub-cellular fractionation allowed for the detection of the polyplexes in different cell compartments, revealing that the T101–PLL–DNA complexes were still entrapped within endocytotic vesicles. To facilitate a sufficient release of the corresponding polyplexes from the endosome, an adenovirus suspension was incorporated into the complexes before incubation. A sufficient transfection efficiency, determined by luciferase expression, was only achieved by the adenovirus-associated polyplexes; without the endosomolytic virus no transfection occurred.

To ensure the co-localization of the adenovirus with the polyplex in the endosome, Wagner et al. formed binary complexes by conjugating the virus to PLL using streptavidin–biotin binding or transglutaminase reaction, followed by the DNA complexation [97]. To achieve active targeting, ternary complexes were prepared by conjugating transferrin to PLL before addition to the binary complexes. The transfection efficiency was determined by measuring the luciferase gene expression in different human and murine cell lines. Investigation of the endosomolytical activity revealed that the ternary complexes with the transglutaminase-conjugated adenovirus had a significantly better transfection efficiency than the complexes together with chloroquine or adenovirus. The specificity of transferrin

targeting was confirmed on adenovirus-receptor lacking K562 cells, showing the highest transfection efficacy of the ternary complexes and possessing both the targeting agent for the transferrin receptor and the endosomolytical properties of the adenovirus.

Leamon et al. tagged folic acid to high molecular weight PLL using PEG spacers with different lengths and investigated the impact on transfection of different cell lines measuring the luciferase gene expression and β-galactosidase expression [87]. The application of PEG with a minimum molecular weight of 3400 has been shown to be most profitable, exhibiting a 10- to 74-fold enhancement of transfection efficiency in the different cell types compared to the polyplexes without the spacer. This finding correlates well with the 'individual' folate receptor expression. Leamon et al. contributed the increased luciferase gene expression to the improved accessibility of the folate ligand for receptor binding.

Many research groups have taken advantage of the endosomolytic properties of PEI to design efficient non-viral vectors with enhanced transfection efficiency facilitated by the accelerated release of the polyplexes from the endosomal–lysosomal compartment. The use of endosomolytic agents, such as adenovirus, could be circumvented, reducing the competitive adenovirus-receptor targeting.

Lee et al. attached biotin-tagged PEGylated EGF non-covalently to the surface of streptavidin-coated PEI–DNA polyplexes, evaluating the effect of EGF-mono- and multi-PEGylation, biotin–streptavidin molar ratio and streptavidin–DNA molar ratio on polyplex stability, complex size and transfection efficiency [168]. Increasing amounts of streptavidin were bound to the PEI–DNA polyplexes by ionic interaction (streptavidin–PEI–DNA). The mono-PEGylated EGF and multi-PEGylated EGF were non-covalently bound to polyplexes with a molar ratio of DNA–streptavidin of 1:100 by biotin–streptavidin interaction using increasing biotin–streptavidin ratios (EGF–PEG–biotin–streptavidin–PEI–DNA) (Fig. 8). The mono-PEGylated EGF conjugated to the polyplex surface formed very stable polyplexes of a size up to 200 nm, while complexes decorated with multi-PEGylated EGF exhibited abrupt aggregation. Transfection experiments were performed on the A431 cell line, which over expresses EGF receptors, applying non-targeted PEI–DNA complexes, streptavidin–PEI–DNA polyplexes and mono- and multi-PEGylated EGF-coated EGF–PEG–biotin–streptavidin–PEI–DNA-complexes, determining the luciferase gene expression. Lee et al. revealed that the PEGylation reduces unspecific cell adhesion, while the conjugation of EGF enhanced receptor-mediated cell uptake, hence increasing transfection efficiency.

Kircheis et al. used the plasma protein transferrin to prevent unspecific interaction with plasma compounds and erythrocytes, demonstrating that transferrin exhibited a shielding effect on PEI 25,000 even without prior PEGylation [167]. The in vitro transfection experiment with K562 cells exhibited a significantly higher transfection efficiency of the transferrin-tagged polyplexes. Motivated by the successful application of the transferrin-tagged PEI–DNA polyplexes in the in vitro experiments, Kircheis et al. investigated the transfection efficiency and organ distribution of transferrin-tagged and non-tagged PEI–DNA polyplexes in an in vivo subcutaneous tumor model. PEI with molecular weights of 800,000 and 25,000 were used for

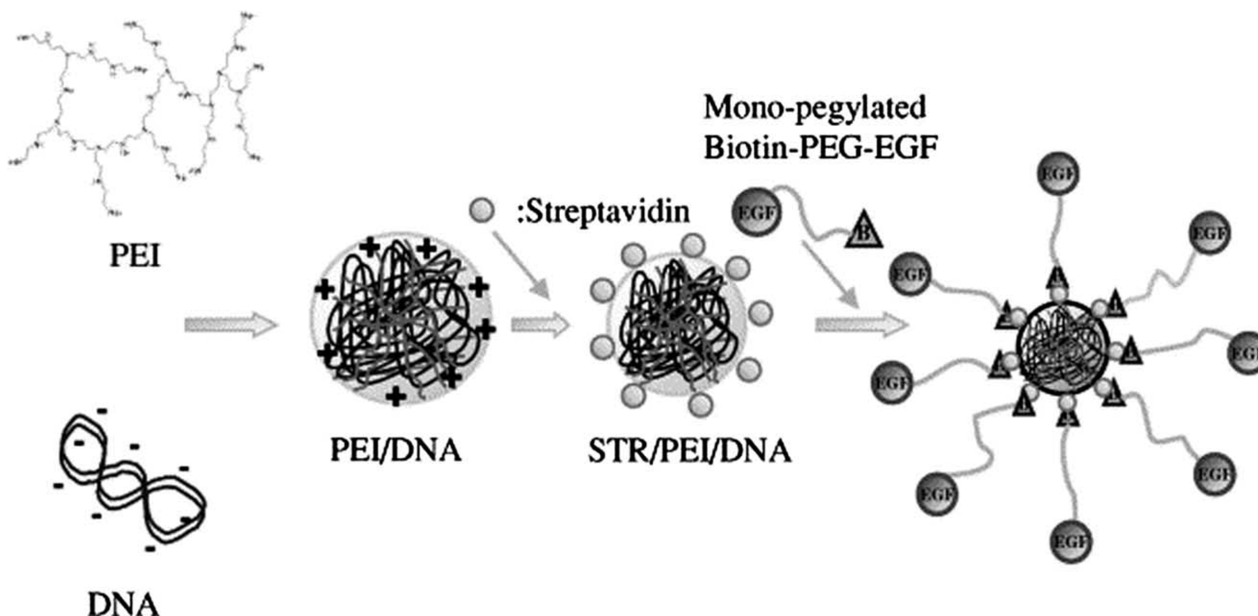


Fig. 8. Schematic illustration of mono-PEGylated EGF–PEG–biotin–streptavidin–PEI–DNA complexes: DNA was condensed with an excess of PEI to form positively charged polyplexes, which, in a second step, have been coated with streptavidin by ionic interaction, yielding neutrally charged polyplexes. Finally biotin-PEG tagged EGF was conjugated to the complexes by non-covalent attachment to streptavidin, decorating the nanoparticle surface with a PEG-shield and the targeting agent. Reproduced from Lee et al. [168].

DNA complexation; the polyplexes were injected into mice and the transfection efficiency was assayed by the luciferase gene expression. These experiments showed that only the charge-shielded formulations of transferrin-incorporating PEI 25,000 and PEG-coated transferrin-PEI 800,000–DNA polyplexes preferentially distributed in the distant tumor, confirming the protective properties of both agents against protein adsorption, enabling the design of long-circulating vectors for gene delivery.

9. Conclusions and future challenges

Biomimetic polymers are used in many different applications ranging from the targeting of single cell types for the delivery of drugs or DNA to modified biomaterials that interact with whole tissues, like implants or prostheses. This review displayed current strategies for biomimetic material design and hopefully gave further ideas for future developments. Selected examples demonstrated the importance of the polymer features to better achieve the intended goals for the biomaterial's main purpose.

The challenges in this field, however, are enormous and frequent, since the knowledge of the whole biological system or even the single cell, which is the main target in all these approaches, is still limited. We must gain a much deeper insight into the biological principles to understand all of the phenomena that are involved in small cellular events, like, for example, the transfer of genes by viruses or the attachment and differentiation of cells on biocompatible surfaces. However, the biomimetic materials introduced in this paper are also useful tools to investigate and elucidate all of these biological principles. Here especially, variable designs allow for the detailed exploration of different signaling molecules, leading to a much broader understanding of cellular communication.

Hopefully, some of the future developments will result in improvements of the therapy of various diseases, which cannot be treated today, and allow for the achievement of better healthcare.

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