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Abstract

The application of biomaterials to regenerate tissues requires research of the interface between the synthetic material and the living tissue. Because biomaterials represent a synthetic extracellular matrix that controls the cell biology by mechanism of cell adhesion, basic mechanisms of cell adhesion are addressed. The technology of designing instructive materials involves chemical modifications by grafting of chemical groups, adhesion ligands and growth factors. Physical characteristics of the materials are created by modifications of the surfaces structure and stiffness of the material. Because stem cells have emerged as promising cells to address the challenge of tissue regeneration the control of stem cells by the characteristic of materials is discussed. Insights into the mechanism at the biointerface that are involved in the regulation of stem cells by materials will advance the development of innovative biomaterials in regenerative medicine.

# Chapter 22
# Biointerface Technology

**Joachim Rychly**

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## 22.1 Introduction: An Historical Perspective

The biointerface is the interface between a nonviable material and the biological tissue or a cell. Mechanisms of the interaction between a material and the biological tissue control the reaction of the tissue and may also determine the fate of the material. The application of materials as medical implants or prostheses has a more than 2000 years history. To replace limbs, eyes, teeth, part of the skull or bone, beside wood or ivory the ancient cultures used mostly different metals. The first polymer as an implant was introduced by the British ophthalmologist Harold Ridley in 1949, when he used poly (methyl methacrylate) to replace a cataracted lens of a patient (Ridley 1952). He made the observation that the eyes of pilots who had shards of canopy plastic in their eyes due to enemy machine gun fire, tolerated

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this material, without ongoing reactions. In addition to implants, also ex vivo devices, like dialysis equipments or heart lung machines form a biointerface, in that case mostly with cells of the blood.

With the introduction of hip implants, vascular grafts or the kidney dialysis first principles of application of medical materials were given by the late 1960s. The principal demand for a medical material was that the interaction of the material with the biological system should not provoke harmful reactions. The term "biocompatibility" originally refers to material characteristics of having no toxic effects or inducing mutagenesis and inflammation. The goal of the early biomaterials was to achieve a biological "inertness". The challenge of the new generation of materials is to create bioactive surfaces that are suitable to specifically control the biology of the tissue. In the field of regenerative medicine the control of stem cell plays a principal role. Therefore, the designing of implant materials is focussed on the question how characteristics of the materials are able to steer all the biological functions of a stem cell, which include self-renewal, differentiation to a specific cellular phenotype, secretion of bioactive factors, or migration. The development of such bioactive material surfaces requires the interdisciplinary collaboration between disciplines of engineering and the life sciences. The progress in this field depends on both the understanding of the biological mechanisms and the development of technological methods. The driving force for the design of bioactive material surfaces is the understanding of the complex mechanisms on the cellular level that determine the regenerative processes in the different tissues of the organism. Therefore, in this chapter first a review of cell biological mechanisms will be given with a focus on the adhesive interactions of cells with the extracellular matrix. These interactions play a key role at the cell-material interface and basically, the aim of material design is to control the cell biology by modifications of the chemical and physical properties of the material surfaces.

## 22.2 Background/Principles

### *22.2.1 Mechanisms of Cell Adhesion*

Cells are regulated by different signals induced by soluble factors, cell-cell contacts and the interaction of cells with the extracellular matrix. Proteins of the extracellular matrix, like collagens, fibronectin, laminin, elastin are secreted by cells and differ in their composition depending on the type of tissue. For example, collagen I is a characteristic matrix component for bone, collagen II for cartilage or laminin for the basal membrane of the epithelium and endothelium. The composition and structure of the extracellular matrix is dynamic and vary which determine its function. This is obvious during processes of the development and tissue differentiation. For example, during the development of branched organs like mammary glad, kidney, gut and lung the branched units are surrounded by a microenvironment that change in composition and spatial distribution over the time (Rozario and Desimone 2009).

The spatio-temporal expression and deposition of extracellular matrix provides instructive differentiation signals. In the mouse development, myogenic differentiation occurs as laminin, collagen IV and entactin expression increases, whereas fibronectin expression decreases (Godfrey and Gradall 1998). Although the control of stem cell differentiation by the extracellullar matrix appears complex, defined matrix molecules induced specific differentiation of stem cells. Embryonic stem cells are normally not competent to differentiate to trophoblastic cells, however on collagen IV but not on laminin, fibronectin or collagen I the cells developed to a trophoblastic lineage (Schenke-Layland et al. 2007). Also directed differentiation of multipotent adult stem cells was dependent on the type of matrix protein. Neural stem cells developed to neurons, astrocytes and glia cells on laminin but not on fibronectin (Flanagan et al. 2006). Osteogenic differentiation of human mesenchymal stem cells was induced on laminin-5, collagen I and vitronectin (Klees et al. 2005; Kundu and Putnam 2006; Salasznyk et al. 2004). The studies also revealed that differentiation to the same phenotype might be differentially regulated by different matrix proteins (Kundu and Putnam 2006). As already mentioned, the extracellular matrix is a highly dynamic structure, which is constantly undergoing remodelling, i. e. assembly and degradation. Experiments using fluorescence time lapse-imaging demonstrated that in a cell culture individual fibrils of fibronectin were stretched and displaced (Sivakumar et al. 2006). Motile osteoblasts actively mediated fibronectin assembly by adding globules of matrix molecules to existing fibronectin fibrils and reorganized the extracellular matrix by shunting matrix material from one location to another or exchanged fibrillar material between fibrils. Remodelling of the extracellular matrix is the result of multiple processes, which requires at least two events: synthesis and proteolytic degradation of the components (Daley et al. 2008). Among the proteolytic enzymes, matrix metalloproteinases (MMPs) play a dominant role in the degradation of the extracellular matrix. Although matrix protein degradation remains a principal physiological function of MMPs, there is evidence that also other substrates, like peptide growth factors, tyrosine kinase receptors, chemokines are a target of MMPs, which indicates a more extensive involvement of MMPs in a variety of physiological processes (Page-McCaw et al. 2007; Stamenkovic 2003). The interaction of cells with the extracellular matrix is mediated by receptors of the integrin family which enable a bidirectional signal transduction (Hynes 2002; Takada et al. 2007). Integrins function as heterodimeric transmembrane receptors consisting of one ß and one $\alpha$-subunit. In human, 18 $\alpha$-subunits and 8 ß-subunits are described, which form at least 24 different receptors (van der Flier and Sonnenberg 2001; Wehrle-Haller and Imhof 2003). The combination of the ß with the $\alpha$-subunit determines the binding specificity for the ECM ligand and a simplified classification into three classes yields a group of integrins, which binds to the RGD sequence (amino acids Arg-Gly-Asp) of fibronectin or vitronectin, receptors which bind to laminin and integrins that bind to collagens (Wiesner et al. 2005). Activation of integrins which induces signal transduction involves conformational changes in the extracellular domain to expose the ligand-binding site (Luo et al. 2007). The conformational changes also enable an increased binding avidity which leads to a clustering of hundreds or thousands integrin interactions with matrix ligands into tightly bound adhesive units (Legate et al. 2009). To connect integrins with the actin cytoskeleton in integrin mediated signal transduction, the formation of adhesion complexes at the interface between cell and substrate plays a dominant role. In these focal adhesions 157 molecules have been identified that are assembled in a "integrin adhesome" and enables signal transduction (Zaidel-Bar et al. 2007). Upon integrin binding to a ligand focal adhesions mature. First nascent adhesions are organized within the the lamellipodium. During maturation the adhesions grow into dot-like structures, which then become elongated to form fibrillar adhesions (Geiger et al. 2001; Wehrle-Haller and Imhof 2002; Zaidel-Bar et al. 2003). This process is facilitated by the α- actinin-actin structures and requires myosin II (Choi et al. 2008). The functions of some of the numerous proteins assembled in focal adhesions have been elucidated. For example, talin facilitates the interaction of integrins with the cytoskeleton by direct binding to the integrin tail, or vinculin plays a role in the formation and growth of focal adhesions (Gallant et al. 2005; Humphries et al. 2007; Zhang et al. 2008). FAK appears to be responsible for turnover of focal adhesions and actin polymerization and is a major component in further downstream signalling events (Zhao and Guan 2009). Downstream, integrin signalling shares common pathways of growth factor receptors, like activation of MAP-kinases (Miyamoto et al. 1996; Moro et al. 1998). Beside the cross-talk between integrins and growth factor receptor pathways, also the physical proximity and lateral collaboration at the cell membrane between integrins and growth factor receptors are important to induce signaling and in consequence a biological function (Schneller et al. 1997).

### 22.2.2 Cellular Mechanotransduction

Cells are able to sense mechanical forces, which control their physiological functions. Physical forces act or are generated at the interface between the cell and the extracellular matrix (Geiger et al. 2009; Mammoto and Ingber 2009; Puklin-Faucher and Sheetz 2009). Therefore, the cellular components that facilitate cell adhesion to the extracellular matrix have a primary role in the cellular sensory machinery and are able to integrate and transduce mechanical signals. Transduction of mechanical forces is bidirectional. While cells are able to sense forces from outside they also generate forces to the extracellular matrix, which is facilitated by the cytoskeleton and regulated for example by actin polymerization (Galbraith et al. 2007; Giannone et al. 2007; Ingber 2006; Kumar et al. 2006). Myosin II is responsible for the contractile nature of the stress fibres to exert forces to the extracellular matrix (Katoh et al. 2001; Peterson et al. 2004). Integrins function as primary sensor and mechanotransducers and facilitate the mechanical coupling between inside and outside the cell (Schober et al. 2007; Wang et al. 1993). Transition of the β integrin subunit from an inactive state to an active conformation can be induced by mechanical forces (Cluzel et al. 2005; Kim et al. 2004; Puklin-Faucher et al.

2006). Mechanical forces directly applied to integrins induce an accumulation of focal adhesion molecules and a direct physical link to the cytoskeleton by immobilizing of signalling proteins, like FAK to the actin cytoskeleton (Cox et al. 2006; Michael et al. 2009; Riveline et al. 2001; Schmidt et al. 1998). To convert mechanical forces into biochemical signalling events, proteins at the adhesive interface are stretched and expose binding sites (Vogel and Sheetz 2009). Vinculin binds to talin rod due to mechanically stretching of the talin molecule (del Rio et al. 2009). Detailed studies revealed that fibrillar fibronectin can be extended by stretch more than eightfold and the mechanically induced unfolding of fibrillar fibronectin alter the displayed binding sites (Klotzsch et al. 2009; Vogel 2006). Fibronectin contains different recognition sites for binding of serum proteins, other matrix proteins, cell adhesion proteins distributed over more than 54 domains that can be switched on and off be mechanical forces (Vogel and Sheetz 2009). Interestingly, the mechanical properties of the fibronectin fibres are regulated, old fibres become more unfolded with age than newly deposited fibres. Further, due to differences in the mechanical strain, fibrillar fibronectin is more unfolded on rigid than on soft substrates (Antia et al. 2008). In addition to a mechano-biochemical conversion near the adhesion site, there is evidence that cells are able to transduce mechanical signals directly to the nucleus because of a structural connectivity between extracellular matrix and cell nucleus (Maniotis et al. 1997; Wang et al. 2009). In this model, the cell is a “hard wired” tensegrity network which refers to a stable interconnected cytoskeleton that resists mechanical stresses and maintain shape stability (Ingber 1997; Stamenovic et al. 1996). The connection between cytoskeletal filaments and the nuclear membrane is facilitated by a LINC complex (linker of nucleoskeleton and cytoskeleton) containing nesprins, sun and lamin proteins (Crisp et al. 2006; Haque et al. 2006). Through lamin A, which binds transcription factors, mechanical forces could directly alter gene expression in the nucleus (Dechat et al. 2008). In addition, mechanically induced expansion or contraction of nuclear pores may alter transport processes into the nucleus (Feldherr and Akin 1990). Such direct force transmission between cell membrane and nucleus may induce a fast induction of gene expression and may explain a rapid increase of calcium in the nucleus (Pommerenke et al. 2002).

### *22.2.3 Interaction with the Extracellular Matrix in the Stem Cell Niche*

The stem cell niche is a specialized microenvironment in various organs which provides an anatomical compartment to maintain a pool of stem cells (Jones and Wagers 2008). The microenvironment, which involves soluble factors, the interaction with other cells and an extracellular matrix, regulate stemness, survival and migration out of the niche (Kolf et al. 2007). To mimic the mechanisms in a niche by bioactive material surfaces, the extracellular matrix is of primary interest. Evidence exists that the composition and mechanical properties of extracellular matrix determines the fate of stem cells in a niche (Daley et al. 2008). It became further obvious that the dynamic remodelling of the extracellular matrix at a specific time and in a tissue-specific manner within a niche function as important switch to trigger stem cell differentiation or mobilization. However, detailed information about a precise role of the extracellular matrix in a niche are rare. Differential expression of integrin-β1 has been observed to regulate cell restriction and mobility of stem cells in the epidermal stem cell niche (Jensen et al. 1999). The fate of neuronal stem cells appeared to be dependent on the expression of β1-integrin (Yoshida et al. 2003). Neuronal stem cell differentiation was accompanied by a decrease in α5β1-Integrin. In a hematopoietic stem cell niche, the matrix glycoptrotein osteopontin plays a role for the hematopoietic stem cells to localize at the endosteal bone surface (Nilsson et al. 2005). In addition, osteopontin was found to suppress hematopoietic stem cell proliferation. Recent studies stressed the assumption that the type of extracellular matrix may determine the direction of stem cell differentiation. Mesenchymal stem cells are localized in a perivascular niche and are exposed to signals from vascular cells (Crisan et al. 2008). On extracellular matrix derived from endothelial cells, mesenchymal stem cells developed markers of endothelial or smooth muscle cells (Lozito et al. 2009).

## 22.3 Technological and Biological Opportunities for Therapeutic Devices

### *22.3.1 Chemical Modification to Control the Biointerface*

#### 22.3.1.1 Modification of Chemical Groups

Chemical as well as physical characteristics of a material control the biological response of the tissue. For tissue regeneration, the key question is that, how the properties of a biomaterial specifically control the different biological functions of stem cells. Different steps of surface designing can generate a bioactive chemistry of a material. First, the chemistry is determined by the pure uncoated material. Next, the chemistry can be modified by grafting chemical groups on the surface, which alter the surface charge and the wettability. More specifically, molecules of the extracellular matrix or peptides which are characteristic of matrix domains and function as binding sites may be immobilized. Last, soluble factors, like growth factor may be incorporated into the material surface, which might by active as solid-phase ligand or which could released by various mechanisms.

Dependent on the application regarding the tissue and function, materials for implants reach from metals to synthetic polymers and natural materials. All these materials differ in the chemistry of the surface. At the interface to a material surface the interaction of the cell is mediated by extracellular matrix proteins. However, prior to a matrix production of the cell, a first adhesive contact of the cell to the substrate can be mediated by a hyaluronan coat of the cell (Cohen et al. 2006; Evanko et al. 2007). The strength of this interaction differs in dependence on the material to which the cell does adhere (Finke et al. 2007). For the subsequent integrin mediated adhesion, adsorption and organization of the extracellular matrix proteins to a material are required. The role of chemical variations of the surface to mediate adhesion dependent stimulation of biological functions of stem cells can be evaluated by generating polymers with different combinations of monomers. Combining 25 different monomers of acrylates to generate 576 polymers allowed a screening to identify materials with the ability to stimulate proliferation and differentiation of human embryonic stem cells (Anderson et al. 2004). Some of the polymers allowed for a high level of cytokeratin positive cells, indicating differentiation to epithelial cells. Interestingly, for some materials proliferation was observed only in the absence of retinoic acid as a soluble factor. This indicates an interaction of signals from soluble factors and the adhesive substrate. A relationship was also established between the ability of the polymers to adsorb fibronectin and cell adhesion (Keselowsky et al. 2003; Mei et al. 2009). Polymers are not only capable to generate different amounts of adsorbed fibronectin, but also induce different activities of fibronectin (Mei et al. 2009). Different techniques have been used to modify the chemistry of a material surface, which involved the use of self assembled monolayers of alkanethiols, silanisation, plasma treatment, radiation grafting (Curran et al. 2005; Keselowsky et al. 2005; Ratner 1995). Grafting of functional groups using glow discharge plasma deposition was also successfully applied to modify titanium surfaces (Nebe et al. 2007). A major challenge of these modifications is the precise control of functional groups. The spectrum of functional groups comprises amino, methyl, hydroxyl, ether, carbonyl, carboxyl and carbonate. Specific alterations of the chemistry were found to guide differentiation and proliferation of mesenchymal stem cells (Curran et al. 2006, Phillips et al.). -$NH_2$ and –SH modified surfaces stimulated osteogenic differentiation, whereas –OH and –COOH modified surfaces promoted chondrogenesis. Under specific culture conditions, -$NH_2$ surfaces enhanced the formation of adipogenic cells (Phillips et al.). Generation of –$CH_3$ groups maintained the phenotype of mesenchymal stem cells (Curran et al. 2006). These biological responses of the cells depend on mechanisms related to changes in the cell-extracellular matrix interaction. Surface chemistry of a material can induce changes in the conformation of fibronectin, which modifies binding of integrins and induces short-term changes in focal adhesion formation (Keselowsky et al. 2004). Generation of –$NH_2$ groups on titanium surfaces using plasma polymerized allyl amine promoted the spreading of osteoblasts (Nebe et al. 2007). Titanium implants are widely used as bone substitutes, e. g. for artificial hip or knee joints. To stimulate bone regeneration at the interface to the bone tissue, titanium coating with calcium phosphate is a suitable approach because of the similarity with the mineral phase present in bone (de Groot et al. 1998; de Jonge et al. 2008). Similarly, calcium phosphate composites are applied as degradable scaffolds to heal bone defects (El-Ghannam 2005). The most successful technique to coat metallic implant with calcium phosphate has been the plasma-spray technique. Because coating must be at least 50 μm thick to completely cover the surface other methods including sol-gel deposition, electrospray deposition, electrolytic deposition have been applied and each has its advantages and disadvantages (de Jonge et al. 2008). Calcium phosphate coatings are described to induce an increased bone-to.implant contact and therefore are regarded as osteoconductive (Barrere et al. 2003; Leeuwenburgh et al. 2006). To see, whether calcium phosphate surfaces may affect bone regeneration, a number of in vitro studies demonstrated that calcium phosphate promote the osteogenic differentiation of mesenchymal stem cells (Cordonnier et al. 2009; Moreau and Xu 2009; Muller et al. 2008; Sun et al. 2008). Although the mechanisms are not known, the observed strong adsorption of fibronectin and vitronectin, as well as a very flat morphology of stem cells on a calcium phosphate surface (Fig. 22.1) could support an osteogenic differentiation (Kilpadi et al. 2001; Walschus et al. 2009).

#### 22.3.1.2 Grafting of Cell Adhesion Ligands

To further specifically control cell adhesion, material surfaces can be grafted with complete molecules of the extracellular matrix or synthetic peptide sequences which represent binding sites of matrix proteins. The best known of these is the RGD peptide containing the amino acids arginine, glycine, aspartic acid which is found in fibronectin, laminin, collagen type IV, tenascin and thrombospondin (Benoit and Anseth 2005; Comisar et al. 2007) and several other adhesion molecules. Structural modifications of the peptides from linear to cyclic RGD peptides are potent

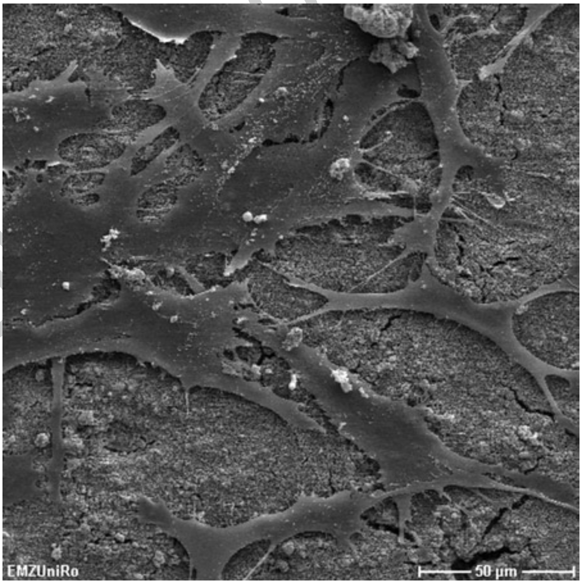

**Fig. 22.1** Mesenchymal stem cells adhere, spread and form a flat morphology on hydroxyapatite coated surfaces

alternatives and can enhance affinity towards a receptor or stimulate cell adhesion (Durrieu et al. 2004; Maeda et al. 1994). In most cases RGD peptides are linked to polymers via stable covalent amide bonds. In this case an activated surface carboxylic acid group reacts with the nucleophilic N-terminus of the peptide (Lin et al. 1994). Alternatively, a coupling is possible in a two step protocol. First, the surface carboxyl group is activated as an ester and followed by coupling the peptide in water (Jo ~~and MIkos~~ 2000). Beside synthetic polymers, other materials, including natural polymers, starch, dextran and inorganic materials have been coated with RGD peptides (Hersel et al. 2003). Among the inorganic materials, titanium and hydroxylapatite were successfully coated with RGD peptides (Fujisawa et al. 1997; Itoh et al. 2002; Rezania et al. 1999). On hydroxylapatite, RGD-peptides were immobilized via negatively charged anchoring groups, like glutamic acid, phosphonates or natural HA-binding amino acid sequences (Gilbert et al. 2000; Hersel et al. 2003; Itoh et al. 2002). To prevent unspecific protein adsorption, grafting of RGD peptides can be combined with passivation of the material suface using e. g. poly(ethylene glycol) (Banerjee et al. 2000; Drumheller and Hubbell 1995). Star-shaped poly (ethylene glycol) prepolymers were used to prevent unspecific protein adsorption and allowed the binding of RGD peptides for specific adhesion of mesenchymal stem cells (Groll et al. 2005). Cell experiments on materials coated with matrix proteins or peptides revealed that integrin mediated interactions with the substrate are complex and require flexible and dynamic mechanisms. Therefore, the introduction of a spacer to bind RGD peptides or matrix proteins improved cell attachment (Craig et al. 1995; Kantlehner et al. 2000). When collagen was immobilized to a polyether ether ketone via glutardialdehyde, osteoblasts did adhere but spread only when polyethylene glycol as spacer was introduced (Fig. 22.2). To further enable a dynamic interaction of cells with the adhesive substrate and remodel the extracellular matrix, materials were crosslinked by enzyme-degradable peptide sequences. The combination of integrin binding and matrix degradation by cellular metalloproteinases allowed the

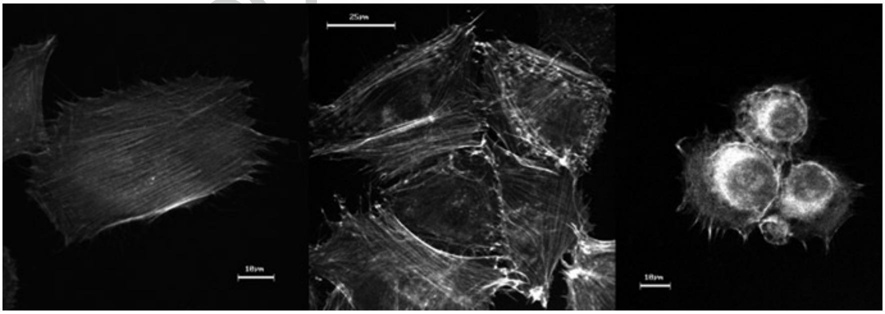

**Fig. 22.2** The mode of collagen immobilization determines the spreading of osteoblasts: *Left*: On cover glass, which was coated by collagen adsorption, cells spread and form actin fibres; *middle*: Cells spread and form actin fibres on a polyether ether ketone (PEEK) surface coated with collagen, which was immobilized by glutardialdehyde (GDA) and polyethylenglycol was introduced as a spacer; *right*: Cells adhere but remain round without formation of actin fibres on PEEK, coated with collagen, immobilized via GDA alone

cells to migrate through a gel, which mimics tissue remodelling (Lutolf et al. 2003a). Enzymatically mediated cell migration has been provided using materials from chemically cross-linked hyaluronic acid (Bulpitt and Aeschlimann 1999; Park et al. 2003). Further, elastase-sensitive sequences were generated by crosslinking elastin-like units which contained the adhesion motif REDV (Girotti et al. 2004). Cleavage of the polymer yielded a bioactive VGVAPG fragment which stimulated cell proliferation. This functionality mimics dynamic processes of the extracellular matrix in vivo, whereby enzymic activities can liberate cryptic binding sites. Although immobilization of matrix-derived peptides demonstrated support of cell adhesion, data of the biological specificity of such approaches are rare (Carson and Barker 2009). When titanium was passivated and grafted with the fibronectin fragment $\text{FNIII}_{7-10}$, this surface enhanced the osteogenic differentiation of mesenchymal stem cells relative to RGD immobilized surfaces (Petrie et al. 2008). This appeared to result from the specific targeting of the β1α5-integrin. The presentation of adhesion peptides in a structural organization that mimic fibrils of the extracellular matrix could further contribute to the biological outcome. RGD peptides in 3D-network of nanofibers promoted the osteogenic differentiation of mesenchymal stem cells (Hosseinkhani et al. 2006). In a three dimensional network of nanofibers the immobilization of the laminin epitope IKVAV induced the differentiation of neural progenitor cells into neurons (Silva et al. 2004).

#### 22.3.1.3 Immobilization of Soluble Factors

The extracellular matrix provides a reservoir for growth factors, which can be released and act as soluble ligands (Hynes 2009). Evidence exists that also matrix-bound growth factors stimulate cell functions via solid-phase signals (Wijelath et al. 2006). Specific binding sites have been detected in the extracellular matrix which can regulate the function of growth factors (Hynes 2009). Therefore, the immobilization of growth factors and other bioactive molecules plays a role in the strategies of designing the surface of implant materials for tissue regeneration (Cartmell 2009; Lee and Shin 2007; Silva et al. 2009). Growth factors bound to biomaterial surfaces may have enhanced activities compared with a soluble form of the factor, as it has been shown for TGF-β1 covalently linked to a polymer and stimulating matrix production (Mann et al. 2001). Different techniques have been applied to tether and control the release of bioactive factors (Place et al. 2009). The easiest way to add soluble factors is to load them into polymer matrix or to adsorb onto a composite (Soriano and Evora 2000; Ziegler et al. 2002). A variety of growth factors have been incorporated into hydrogels during the formation of the material in aqueous solution (Kanematsu et al. 2004). To tune the release of soluble proteins, the cross-linking density of the polymer can be modified (Hiemstra et al. 2007). bFGF could be released quantitatively from such hydrogels in 28 days. These techniques basically rely on the passive diffusion of growth factors from the matrix. Another strategy for protein release relies on a mechanical-responsive system (Augst et al. 2006; Lee and Mooney 2001). Many tissues, such as vasculature and musculature are mechanically dynamic. Mechanical compression could release factors from a material. Using a VEGF-containing alginate-hydrogel, it was shown that exposing mechanical strain to the hydrogel increased the release of VEGF (Lee et al. 2000). After implantation in mice, this mechanically induced release increased collateral vessel formation. Adding growth factors to ceramic materials is very convenient, because ceramics have a high affinity for proteins (Ziegler et al. 2002). Growth factors, such as TGF, FGF and VEGF were loaded to ceramics just by adsorption. The release patterns of most loaded ceramics seem to consist of an initial burst release of not bound protein followed by a second release dependent on the material/protein interaction (Habraken et al. 2007). Loading of calcium phosphate cements with growth factors was performed just by adding the protein to the liquid hardener, thereby distributing it equally through the cement. Bovine serum albumin can be used as carrier solution for growth factors to control the release of factors from the cement (Blom et al. 2002; Ruhe et al. 2006). Several in vivo studies proved the beneficial effects of growth factor loaded calcium phosphate scaffolds (Jansen et al. 2005; Kroese-Deutman et al. 2005; Ruhe et al. 2004; Seeherman and Wozney 2005).

More precise, growth factors can be immobilized to a material surface by covalent binding. This can be achieved by reacting of the side chains of polymers with amino acids of a growth factor. Several growth factors have been covalently linked to polyethylene glycol, including TGF, EGF, bFGF (Bentz et al. 1998; DeLong et al. 2005; Kuhl and Griffith-Cima 1996). To control the release of covalently attached growth factors by the cells, synthetic hydrogels have been generated which contained protease sensitive binding sites (Lutolf et al. 2003a; Zisch et al. 2003b). In this case the hydrogels are prepared with functionalities of natural extracellular matrix, i. e. the ability to mediate adhesion and to respond to proteolytic degradation by enzymes, such as metalloproteinases which are secreted by cells. As structural building blocks, end-functionalized polyethylene vinylsulfone chains were used with thiol-bearing peptides. Cross-linking occurred by incorporation of bis-cysteine peptides, which can be cleaved by proteases. Growth factors, like VEGF and BMP were bound to these structures and could be delivered on cell demand (Lutolf et al. 2003b; Zisch et al. 2003a). Using this approach, an active liberation of VEGF was confirmed which resulted in a remodelled vascularized tissue, when the matrix was implanted subcutaneously in rats (Zisch et al. 2003a). Similarly, bone regeneration was demonstrated in a critical size defect by cell-mediated proteolytic release of BMP from a matrix (Lutolf et al. 2003b). A further more natural mechanism of the control of growth factor binding, modulation and release is the attachment of glycosaminoglycans to a material surface. These complex molecules have a tissue specific distribution and multiple physiological functions (Raman et al. 2005). Their sulphation patterns determine the specific interaction with proteins. One example is the binding of bFGF to heparin. Heparin has been widely incorporated into scaffolds to bind and release bFGF (Sakiyama-Elbert and Hubbell 2000; Zhang et al. 2006).

As demonstrated recently, the physiological effect of growth factors can be mimicked by designing of a modular peptide (Lee and Murphy). This peptide contained a BMP-2 derived peptide sequence and hydroxyapatite-binding sequences inspired by the N-terminal alpha-helix of osteocalcin. The multifunctional fusion protein can bind to hydroxapatite coated surfaces or bone structures and exert BMP activity. When this peptide was presented to mesenchymal stem cells, both immobililized or in solution, the construct was capable to promote the osteogenic differentiation of the cells (Lee and Murphy).

Microspheres with encapsulated or surface bound growth factors present a system to persist and deliver growth factors at the target site (Arras et al. 1998; Cleland et al. 2001; Park et al. 2009). For the fabrication of biodegradable polymer microspheres polyester like polylactide (PLA) and poly(lactic-co-glycolic acid) have been used. Applying a double emulsion technique, growth factors, such as bFGF, VEGF have been mixed into the particles (Perets et al. 2003). The loaded microspheres were incorporated into an alginate matrix or hydrogel. This approach enables the delivery of two or more growth factors with distinct kinetics. Microspheres containing PDGF were mixed with VEFG prior to processing into scaffolds, which resulted in a rapid release of VEGF and a slower, more even distribution of PDGF. When the scaffolds were implanted into rats, the distinct release kinetics of the growth factors stimulated the formation of a mature vasculature (Richardson et al. 2001).

## *22.3.2 Physical Modification to Control the Biointerface*

### 22.3.2.1 Structural Organization of the Surface

The structure of a material surface can be categorized into topography and chemical patterning. The topography reflects the roughness of a surface which can be designed by ridges and grooves or by evenly or randomly distributed pits or protrusions. Chemical patterning is achieved by the spatial organization and immobilization of molecules in controllably size and position, mostly to control cell adhesion (Lim and Donahue 2007).

For clinical application of titanium implants different techniques have been used to roughen the surface, which include blasting, etching, and oxidation. A huge number of experimental data demonstrate that a rough implant surface has a beneficial effect on the bone response (Wennerberg and Albrektsson 2009). This concerns roughness in the micrometre level, whereas little is known about the effects of topographies in the nanometre level in vivo (Wennerberg and Albrektsson 2009). When testing the cell behaviour on topographies the scale plays an important role. It became obvious that cells are able to sense the micro- and nanoscale topography and react with bridging of grooves or conforming the surface structure (Millette et al. 1987; Teixeira et al. 2003; Walboomers et al. 1999). The behaviour of the whole cell due to a topography was correlated with an orientation of the cytoskeleton and the alignment of focal adhesions (Dalby et al. 2003; Dalby et al. 2002). In addition to structural changes in the organization of cellular components, functional consequences have been observed. Osteoblastic cells expressed a higher RNA level of osteopontin and osteocalcin when cultured on a surface with grooves than on a flat surface (Matsuzaka et al. 2004). Apparently, a defined size of pits or grooves is important on a structured surface. As shown, osteoblastic differentiation measured by the activity of alkaline phosphatase was stimulated more on 11 nm islands than on 85 nm islands (Lim et al. 2005). Similarly, also cell proliferation depends on defined surface structures. Progenitor cells displayed a higher proliferation rate on 5–40 μm diameter posts compared with cells on a smooth surface (Mata et al. 2002). In addition to the size of posts created on a surface the organization of a pattern controls the function of cells. When mesenchymal stem cells were cultured on disordered dots with nanosize the cells were induced to express osteocalcin and osteopontin in the absence of osteogenic supplements, demonstrating the stimulation of osteogenic differentiation (Dalby et al. 2007). In comparison, when the same nanofeatures were symmetrically organized, the cells did not express osteogenic proteins.

Although experiments are rare which demonstrate that a defined topography, regarding topographic size, shape or uniformity control a specific function of stem cells, it is obvious that micro- and nanostructured surfaces stimulate various collective cell functions (Lim and Donahue 2007).

Chemical patterning which generates precisely defined micro- or nanometer-areas for cell adhesion can be achieved by lithographic techniques (Nie and Kumacheva 2008). These techniques involve photolithography and printing techniques. Printing methods can be classified into techniques which involve the contact of a stamp with the substrate and methods which directly transfer “ink” to the substrate. Dip-pen nanolithography represents a relatively new direct writing technique, using the tip of an atomic force microscope to form a liquid meniscus between tip and substrate, and as a result of this procedure the ink molecules are transferred to the underlying substrate by chemical or physical adsorption (Piner et al. 1999). Micropatterning allows the spatial control of adhesion of the whole cell. By restriction of cell spreading the shape of cells can be controlled. Using mesenchymal stem cells, it was demonstrated that cell shape commits the direction of differentiation (McBeath et al. 2004). More rounded cells differentiated to adipocytes, whereas flat cells became osteocytes. The authors revealed that induction of mechanical tension of the cytoskeleton, which correlates with stress fibre formation and is mediated by the activities of RhoA and Rho kinase (ROCK) induces osteogenic differentiation. Blocking of RhoA and ROCK activities stimulated the adipogenic differentiation. By generating fibronectin lines in the nanoscale which altered the cell morphology, the proliferation of embryonic stem cells was stimulated, which depended on an altered organization of the cytoskeleton (Gerecht et al. 2007).

In addition to control of the entire cell shape by adhesion patterns, the sensing of nanoscale adhesion sites by cells controls integrin mediated signal transduction and in consequence influences differentiation and proliferation. For example, the precise spacing between nanotopographic features of RGD-peptides for cell adhesion can modulate the clustering of integrins. A minimal distance of 58 nm between adhesive dots was required for integrin clustering, formation of stable focal adhesions and cell spreading (Arnold et al. 2004; Cavalcanti-Adam et al. 2007). The formation of a molecular gradient of the ligand spacing from 50 to 80 nm revealed that cells are able to sense the small differences in ligand spacing (Arnold et al. 2008). Differences which are little as 1 nm seem to affect cell polarization and migration.

#### 22.3.2.2 Mechanical Characteristics of the Surface

Mechanical stimuli represent regulators of development and function in many tissues. It is generally accepted that the structure of the various tissues reflect the acting forces, which specifically control the physiological processes. In some cases, tissues are heterogeneously organized into mechanically distinct zones, for example the superficial, radial and tight zones of cartilage. Therefore, implant materials must provide some level of physical support to assist tissue function. Engineering strategies have been developed to steer the viscoelastic properties of implant materials, for example by cross-linking of polymers. Highly elastic gels of cross-linked hyaluronic acid with controllable viscoelasticity were generated for tissue engineering of vocal folds (Sahiner et al. 2008). For tendon repair, gels were combined with a type I collagen sponge to optimize the stiffness of the material, which was successfully applied in a patellar tendon model (Butler et al. 2008). Findings in several cell types provide evidence for the importance of the substrate stiffness as a physical signal for cells (Georges and Janmey 2005). Early experiments demonstrated that differentiation of mammary epithelial cells increased when grown on soft collagen gel substrate, as opposed to tissue culture plastic (Emerman et al. 1979). Neurons preferentially branched on soft tissues compared to stiff surfaces (Flanagan et al. 2002). Although in most of these studies, the influence of different mechanical properties is difficult to separate from the type and density of the chemical ligand, it is obvious that stiffness of the substrate plays a role in tissue development. The role of substrate stiffness in the context with regenerative processes was emphasised by the fundamental finding that stem cell lineage specification can be determined by mechanical properties of the substrate (Engler et al. 2006). Mesenchymal stem cells were grown on polyacrylamide gels with varying compliance. These experiments convincingly demonstrated that the stiffness of the material defines the differentiation lineage (Discher et al. 2009; Zajac and Discher 2008). Soft substrates which mimic the mechanical properties of brain stimulated the neurogenic differentiation, intermediate stiffness leads to muscle cell differentiation and stiff substrates where found to be osteogenic. Similar experiments using adult neural stem cells have shown that softer substrates provoked neuronal differentiation, whereas stiffer materials induced the formation of glial cells (Saha et al. 2008). The mechanical properties of the substrates were also found to control the self-renewal of stem cells. Adult stem cells from skeletal muscle tissue revealed increased cell proliferation with rising stiffness of the matrix (Boonen et al. 2009). Mesenchymal stem cells were kept quiescent on a gel that mimicked the softness of bone marrow. In contrast stiffer substrates induced the entry of these cells into the cell cycle (Winer et al. 2009). The cells maintained the multilineage potential and could be differentiated both to adipocytes and osteocytes. These experiments provided evidence of mimicking the functional capacity of a bone marrow niche by tuning the mechanical properties of an artificial substrate. In addition to the control of proliferation and multipotential differentiation, sensing of substrate stiffnesses enables cells to migrate from soft to stiffer matrices, which appears of importance for stem cell translocation to sites of tissue regeneration (Gray et al. 2003; Kidoaki and Matsuda 2008). This phenomenon was termed "durotaxis" (Lo et al. 2000).

## 22.4 Applications for Therapeutic Devices

Progress in biomaterials design and engineering are converging to enable a new generation of instructive materials to emerge as candidates for regenerative medicine. The aim of the design of current biomaterials is to regulate tissue regeneration by modulating direct or indirect chemical and physical control over transplanted or host cells. The dilemma is that to influence cell behaviour, biomaterials must provide complex information (Place et al. 2009). Tissue engineered skin equivalents have been introduced into clinical practice in 1997. Since then tissue engineered devices have been in clinical trials or already approved as therapies for tissues including cartilage, bone, blood vessel and pancreas. However, over-engineered devices make their translation to clinical use unlikely. The reconstruction of entire organs has largely given up and changed to smaller goals. For example, clinical advance in cardiac repair focus on coronar arteries, valves and regeneration of the myocardium. In principle, the aim is to develop synthetic materials that establish key interaction with cells that stimulate the innate organization and self-repair of the body.

## 22.5 Barriers to Practice and Prospects

A major hurdle for the progress in the application of biomaterials in the field of regenerative medicine lies not in the biomaterials but in stem-cell biology. The advancement of basic research in stem cell biology represents the driving factor for the development of biomaterials to regenerate a specific tissue. Current trends suggest that biomaterial development will continue to create more life-like multifunctional materials that are able to simultaneously provide complex biological signals (Chan and Mooney 2008; Howard et al. 2008). Much can be learned from the mechanisms that regulate cell fate in the stem cell niche. For example, the adhesion molecules that contribute to asymmetric stem cell division have begun to identified within the niche environment of hair follicle, intestinal epithelial, spermatogonial stem cells (Kanatsu-Shinohara et al. 2008; Ohyama et al. 2006; Tanentzapf et al. 2007). In addition to the general control of stem cell function, there is growing interest in the dynamic nature of stem cell niches which can change properties under certain conditions (Adams and Scadden 2008).

## 22.6 Conclusions and Future Challenges

Chemical and physical characteristics of biomaterials are able to control the biology of stem cells and significant advances have been gained in in vitro studies. By controlling the properties of biomaterials we may further improve the regulation of stem cell in a bioartificial system. Although stem cell function is regulated by a set of different signals from the environment, the control of the extracellular matrix has proven a valuable tool to guide the development and commitment of stem cells. The challenge is to engineer an artificial extracellular matrix, which is capable to directly control the behaviour of stem cells. In addition, the outcome of growth factors administration can be improved enormously with the use of slow-release constructs. A further step in the generation of bioactive materials will be the design of heterogeneous constructs and even complex organs, which will require both more insights the mechanisms of cell and developmental biology as well as innovation in biomaterial research.

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