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Review

Receptor-ligand interactions: Advanced biomedical applications

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ABSTRACT

Receptor-ligand interactions (RLIs) are at the base of all biological events occurring in living cells. The understanding of interactions between complementary macromolecules in biological systems represents a high-priority research area in bionanotechnology to design the artificial systems mimicking natural processes. This review summarizes and analyzes RLIs in some cutting-edge biomedical fields, in particular, for the preparation of novel stationary phases to separate complex biological mixtures in medical diagnostics, for the design of ultrasensitive biosensors for identification of biomarkers of various diseases at early stages, as well as in the development of innovative biomaterials and approaches for regenerative medicine. All these biotechnological fields are closely related, because their success depends on a proper choice, combination and spatial disposition of the single components of ligand-receptor pairs on the surface of appropriately designed support.

Contents

1. Introduction

Highly specific molecular recognition is one of the fundamental principles of functioning of living systems being involved in all the most important biological processes, such as hormone-receptor and antigen-antibody interactions, enzymatic reactions, transmembrane transport of various substances, among others. From a general point of view, molecular recognition is a key concept of supramolecular chemistry. It can be described as a process of high affinity interaction and selective binding of a ligand by a receptor [1]. Usually the ligands are relatively simple substances of various nature, for example, peptides, proteins, short DNA or RNA, steroids, parts of virus or bacteria or artificial compounds and drugs. On the contrary, receptors are water-soluble, membrane-anchored or membrane-embedded macromolecules with a

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complex 3D structure [2–5]. Ligand-stimulated alterations of cell receptors' state result in physiological responses, which constitute the biological activity of various biomolecules, as well as the action of pharmaceutical drugs [6,7].

Receptor and ligand form a complementary pair with relatively strong non-covalent bonds formed by Van der Waals forces, hydrophobic, π-, ionic or electrostatic interactions. The type of this interaction depends on the structural and energetic compatibility of the biopartners [8]. Receptor-ligand pair formation is a complex process and may include different stages. For protein-protein interaction, that is one of the most complicated cases of receptor-ligand pair formation, it consists of (1) *primary recognition* of receptor by corresponding ligand at the large distance by means of electrostatic forces, (2) *orientation* and change of structural conformation in order to achieve the proper interface contact, and (3) *physical binding* of two molecules [9] (Fig. 1).

Sometimes the ligands contain multiple recognition sites and thus might bind to clustered receptors. This multivalent binding can drastically enhance the affinity of receptor-ligand interaction [10,11].

Upon receiving the signal generated by binding of a ligand to its complementary receptor, a cascade of chemical reactions occurs. The high affinity receptor-ligand pairs form in the cell an extremely complex network of interacting molecules [12]. One signaling molecule can activate multiple downstream signaling pathways through different receptors, while one receptor might propagate various signals depending on ligand partners.

Ligand-receptor interactions are fundamental for the communication of a cell with its neighbours and the whole organism and initiate not only dynamic processes, such as proliferation, apoptosis, movement, but also maintain cell homeostasis and equilibrated functioning of all cell systems.

The research carried out during the last decades allowed noticeable improvement of our knowledge concerning the mechanisms of interaction between biomolecules, that was caused by huge progress in genetic engineering, X-ray crystallography, computational and various physicochemical techniques [13–15]. The information, which can be gathered by identification and characterization of RLIs, is of paramount importance for discovering new receptors and ligands, understanding pathogenesis and molecular mechanisms of endogenic ligands' and pharmaceutical drugs' action, as well as for the development of novel approaches to the treatment of a wide spectrum of various diseases. Various ligand-receptor pairs and RLIs of living cells can be used to create so-called smart biomaterials for a plenty of physicochemical and biomedical applications in the fields of life sciences, industrial biotechnology and pharmacology, tissue engineering, design of highly sensitive diagnostic tools, etc. In particular, three nanotechnological areas have been influenced to the major extent by the advancing of our knowledge on peculiarities of RLIs and discovery of new cell signaling pathways: 1) high affinity separations; 2) construction of ultrasensitive biosensors; 3) design of biomaterials for tissue engineering and regenerative medicine. Though being very far at a first glance, all these nanotechnological fields, are closely related. Indeed, all of them are based on the receptor-ligand interactions, where one of the components of ligand-receptor pair is immobilized on a solid support, as for stationary phase in affinity chromatography and sensing surface of biosensors, or it can be located on bioactive surfaces or live cell membranes in tissue engineering. In all three cases the choice, combination and disposition of the components of receptor-ligand pairs on the interfaces are of crucial importance for the desired outcome. In the case of biomaterial design, the target is not only to form a high affinity ligand-receptor complex, but also to create a bioactive surface or bulk material in order to induce a controllable response in biological environment, which can result in regulation of cell adhesion, migration, proliferation, etc. Thus, the creation of biomaterials aimed to be used in living systems is the highest and the most complicated level of bionanotechnology, where a number of various parameters have to be taken into account, including the interaction with neighbouring cells and tissues, as well as the cross-talk with other ligand-receptor signaling queues.

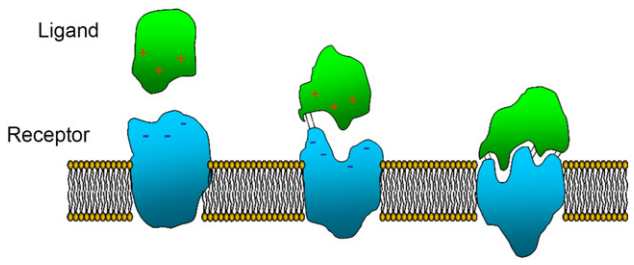

Fig. 1. Ligand-receptor complex formation (from left to right): primary recognition, orientation and physical binding.

This review represents an attempt to summarize and classify the huge amount of more and more sophisticated approaches of bionanotechnology from the point of view of ligand-receptor interactions lying on their base. We moved from the simplest (affinity chromatography) to the most complicated (advanced biomaterials) field to show how to govern *in vitro* and *in vivo* behaviour and functionality of modern artificial media using the unique principle of biocomplementarity. This knowledge is fundamental for the creation of the artificial constructs aimed to be incorporated into the living systems without affection of the metabolic processes. Here, we tried to summarize and analyze various types of ligand-receptor pairs used in different fields of bionanotechnology and biomedicine and to highlight the importance of RLIs for their future development.

2. RLIs in bioseparation

2.1. Monolithic stationary phases

The discovery of the principle of highly specific molecular recognition and the understanding of the fundamentals of interaction between biomacromolecules stimulated the development of a number of highly efficient and fast methods for separation of complex biological mixtures, as well as for purification of low-abundant biomolecules. Affinity chromatography is one of the most powerful tools applied for analysis and purification of biological products of different classes, such as enzymes, hormones, receptors, recombinant proteins, DNA, etc., where one of the components of the affinity pair, i.e. ligand or receptor, is immobilized on a stationary phase, whereas another one is dissolved in a flowing liquid. Among three above mentioned bionanotechnological fields it represents the easiest case, since the modification of solid support with the components of ligand-receptor pairs is aimed to achieve only a selective adsorption followed by a desorption of targeted molecules (Fig. 2).

A plenty of various bioseparation approaches have been developed in the last decades due to the improvement in our knowledge about the peculiarities of ligand-receptor complex formation, the strength of non-covalent bonds inside the complex and how to arrange in space the components of receptor-ligand pairs in order to achieve the highest affinity for target molecules [16–18].

The efficiency of affinity chromatography depends not only on the proper selection and spatial disposition of the components of the ligand-receptor pair, but it is also closely related to the characteristics of solid support and the mechanism of mass transfer between liquid and solid phases. Therefore, a big attention has been paid to the development of new generations of stationary phases, where the influence of solid support on kinetics and thermodynamics of ligand-receptor interaction was minimized [19]. At present, a variety of sorbents have been proposed for the immobilization of biospecific ligands, for example, natural polymers (cross-linked highly swelling agarose and dextran), synthetic polymers (polyacrilamide), inorganic porous supports (silica gels, macroporous glasses), as well as different composites. Among these conventional materials monolithic stationary phases, both organic and inorganic (mostly silica-based), are of particular interest because of

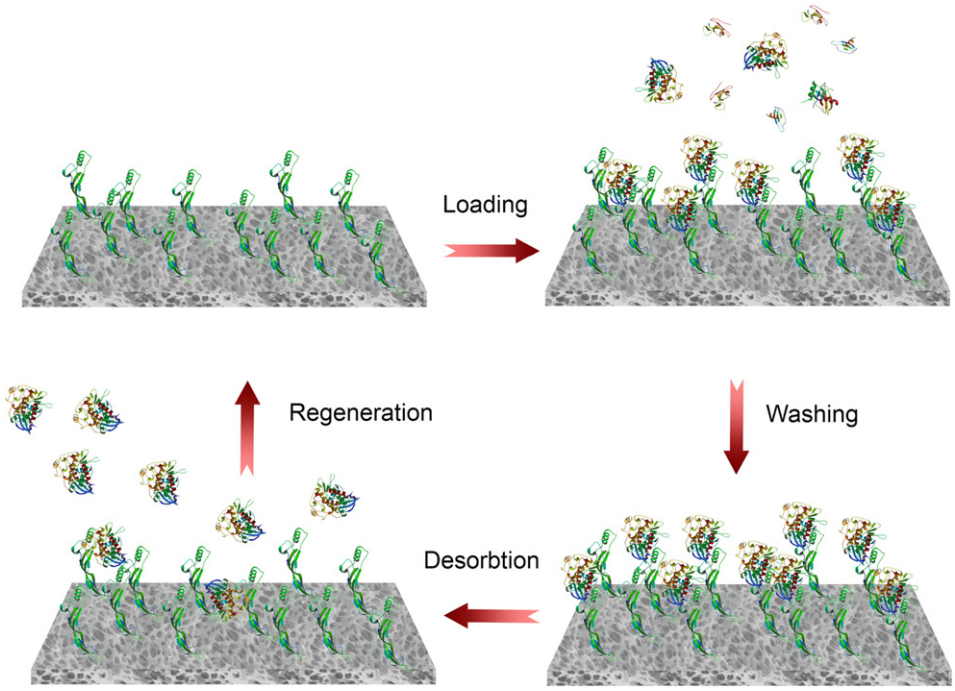

Fig. 2. A scheme of affinity bioseparation process.

the absence of void volume (the space between packed particles) and, therefore, improved fast convection mechanism of mass transfer instead of slow diffusion taking place in conventional packed columns [20–24]. Polymeric macroporous monoliths, directly synthesized in a separation device by free radical copolymerization of various monomers in presence of pore forming substances in the shape of ultra short columns (CIM disks or membranes), rods, tubes, microfluidic chips, microarray platforms, etc., showed to be very effective for fast bioaffinity separations/detection of various complex biomolecules, such as glycoproteins, immunoglobulin G, trypsin, DNA, various peptides and even entire viral particles and parts of cells. [25–31].

The adsorption sites in the monolith structure can be introduced by direct synthesis on the surface (divergent approach), or after one- and multi-step functionalization by means of various chemical reactions (convergent modification) [32]. Such systems, for example, were successfully used for the isolation of recombinant tissue plasminogen activator (TPA), where both direct solid phase peptide synthesis on the pore surface of glycidyl methacrylate-co-ethylene dimethacrylate (GMA-EDMA) monolith and covalent attachment to the same epoxy-bearing support of a series of the same pre-synthesized peptide ligands recognized by TPA were studied [33]. All peptides, chosen from the structure of TPA binding site of its natural substrate plasminogen, showed very high affinity to the protein of interest independently on the way of monolith modification, and were proposed for *on-line* detection of accumulation of recombinant TPA in a cell supernatant (monitoring of its biotechnological production), and further for highly selective and highly productive isolation of final product directly from a complex medium in a scaled-up mode.

In another work immobilization of the peptide His-Trp-Arg-Gly-Trp-Val on the tentacle-type monolith was used for the high-performance immunoglobulin G purification [26]. To this purpose glucose-based long polymer chains were grafted on the inner pore surface of highly porous monolithic support with their further modification with the peptide. Such immobilization allowed distinguishing the enhanced column permeability, structure stability and higher peptide peptide density, respectively to the un-grafted monolith, and to increase the adsorbing capacity of immunoglobulin G up to 101.8 mg/ml.

An interesting method for selective glucose oxidase extraction was developed by modification of hydrophilic polymeric monoliths on the base of polyether ether ketone (PEEK) with lectin from *Lens culinaris* hemagglutinin [30]. It allowed reaching the column binding capacity up to 2.2 mg/column of glucose oxidase.

Short monolithic columns showed to be also very promising for isolation and fine purification of viruses and viral vaccines, when usual chromatographic methods had low efficiency because of the restricted diffusion mobility of relatively big viral particles. For example, hemagglutinin-sialic acid ligand-receptor pair was used for the specific binding both of virus model and the live virulent influenza virus A [34]. Sialyl derivatives were attached to the monolith surface directly or after its modification with various macromolecules, such as protein ceruloplasmin, synthetic biocompatible copolymer of 2-deoxy-*N*-methacrylamido-D-glucose (MAG) with 1-vinyl-2-pyrrolidone (VP) and diethylacetal of acrolein (DAAc), as well as polysaccharides chitosan and heparin. It was shown a complete functional and sterical equivalence of synthetic virus-like model, based on latex nanoparticles and modified by hemagglutinin, and the real virus, which can facilitate the process of development of the stationary phases for purification of vaccines for the treatment of diseases caused by highly infectious viruses. Similarly, streptavidin functionalized monolith was used for the capturing and purification of Moloney Murine leukaemia virus, commonly used in the preparation of gene therapy vectors. It was achieved a 425-fold increase in the virus titre [35].

A simple method of controlling the efficiency of ligand-cell receptor interaction using affinity chromatography on macroporous monolith has been developed by modeling cell adhesion on a solid support with subsequent cell proliferation and bone tissue formation [36]. The biospecific peptide GRGDSP played a role of a model ligand immobilized on the solid support, whereas cells were simulated by polymer (polystyrene) microparticles bearing complementary peptide EDYPVDIYYLMDLSYSMKDD (a fragment of RGD-binding site of integrin receptor). Thus, the monolithic ultrashort column represented a simplified model of a scaffold with biospecific properties. The data obtained in the work indicated a high specificity of biological pairing, which was later confirmed by the results of cell culture experiments [37].

The use of monolithic supports may be a very promising approach for the quality control and quality assurance in the industrial production and purification of various therapeutic substances, as it was shown, for example, in 38. The immobilization of peptide-N-glycosidase F (PNGase F) on GMA-EDMA monolithic polymer allowed efficient release of N-linked glycans from various glycoproteins, such as ribonuclease B, chicken albumin, human immunoglobulin G. In this way it was obtained an enzymatic reactor, that was coupled with MALDI-ToF MS system and used for the fast glycan analysis. The immobilization of trypsin and endoproteinase LysC, allowed reducing time of proteolysis of various proteins, such as cytochrome C, bovine serum albumin, human immunoglobulin G, for their characterization and identification in quality control [39]. The same approach was used for the immobilization of trypsin on poly(acrylamide-*co*-methylen-*bis*-acrylamide) monolith and allowed shortening the digestion time from 24 h to 20 s [40].

Thus, modern fast affinity chromatography using monolithic stationary phases allows not only efficient isolation of target biomolecule, but can serve as simple and robust method for quantitative evaluation of ligand-receptor pairs aimed to be used for the construction of efficient systems of different biomedical applications.

2.2. Artificial receptors: molecular imprinted bioadsorption sites

Despite a high binding efficiency and selectivity of the molecular recognition of natural ligand-receptor pairs, the significant efforts have been applied to construct artificial receptors, which lack many drawbacks of original systems, such as high thermal sensitivity and low stability at extreme pH values [41,42], as well as the risk of a change of the native biomacromolecule conformation after covalent immobilization, that is especially important for proteins.

Water compatible molecular imprinted polymers (MIPs) have emerged as artificial "macro" receptors with specific recognition sites for the detection of various substances – from small organic and inorganic molecules to large proteins, and are facing various analytical applications [43]. In this relatively simple technique a 3D polymer matrix is formed around target (template) molecules (Fig. 3).

Upon their removal, the cavities with precisely defined shape can recognize and then bind the initially used template. MIPs have a number of advantages over the other conventional liquid phase separation approaches due to their high mechanical, thermal and chemical robustness and resistance, as well as low cost and easy preparation. A combination of above mentioned monolith technology with MIP approach has further improved the selectivity, robustness and performance of stationary phases for solid phase extraction of target compounds and separation of complex biological mixtures [44–46].

High selectivity and sensitivity, as well as increased stability of molecularly imprinted polymers make them a powerful tool for the creation of the detecting unit of chemosensors, that can be used in the corresponding structural elements of biosensors (see part 3. RLIs in biosensor design) [47]. Indeed, bioreceptors often suffer from many drawbacks, such as a risk of the degradation in the inappropriate conditions (pH, temperature, organic solvents). High cost of the bioreceptors can be also a limiting factor [48]. Thus, MIPs can be a convenient alternative for the natural receptors in the construction of the sensing devices. In this case, the ligand binding cavities of the receptors are mimicked by the artificial smart biomaterials [49].

To the present time a number of works aimed to improve various steps and sensitivity of MIP technique has been published, both for chemosensor design and (to the less extent) for the preparation of the high affinity sorbents [50]. Despite still limited commercial application, a lot of very interesting and promising examples of the use of the molecularly imprinted polymers can be found in the literature.

For instance, a water compatible MIP to detect 3-nitro-L-tyrosine (a marker of oxidative stress) in urine samples was prepared by bulk polymerization of methacrylic acid and acetonitrile as porogen [51]. A solid-phase extraction of the biomarker allowed reaching the detection limit of 0.7 μg/ml in the linear range of 2.5–55.0 μg/ml with high recovery yield. A similar approach has been used for detection and quantification of trifluorperazine (TFP) in biological liquids [52]. In this case, a 3D matrix with TFP-binding cavities was prepared from methacrylic acid and porogenic chloroform and showed very high sensitivity to the drug. A rapid and simply automated method of insulin detection was developed by combination of MIP cartridge for the solid phase on-line extraction of hormone with HPLC analysis. A high recovery and good selectivity to insulin with low detection limit up to 0.03 ng/ml were thus achieved [53].

A capacitive chemosensor with ultrathin artificial receptor layer was designed for highly selective creatinine detection. In this case, an alkane thiol modified gold electrode surface was used for the acrylamidomethyl propanesulphonic acid monomer polymerization in presence of creatinine template [54].

An interesting method for the MIP preparation was used in the design of chemosensor for the detection of pro-inflammatory immune response biomarker neopterin [55]. Two different functional monomers were copolymerized in presence of neopterin template. One of them contained bithiophene fragment derivatized with boronic acid for covalent bonding of diol moiety of neopterin, whereas another one had cytosine fragment for the formation of the complex with the pterin moiety of the ligand by Watson-Crick type hydrogen bonding. This molecularly imprinted polymer allowed neopterin determination in the range of 0.15–2.5 mM with a detection limit of 22 μM and was suitable for clinical analysis. Analogously, the bithiophene boronic acid derivative containing monomer was used for the complexation of D-arabitol for its determination in urine during a fungal infection [56].

A metal coordination bond and hydrogen bonding in the recognition cavity of the molecularly imprinted polymer was used for the preparation of the sorbent for solid phase extraction for the selective determination of the trace amounts of α-amantin in serum of the patients with the poisoning with Amanita fungi [57]. In this case it was possible

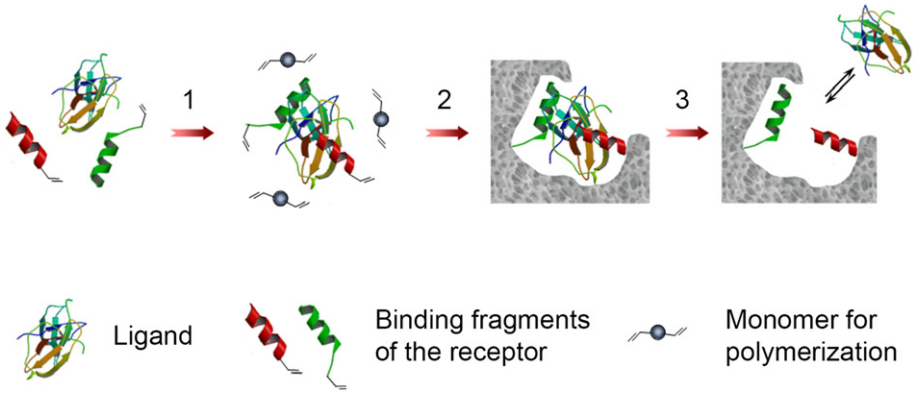

Fig. 3. A scheme of MIP preparation: (1) formation of a pre-polymerization complex with a template and addition of functionalized monomers, (2) polymerization, (3) ligand removal.

to reach the limit of detection of 3.0 ng/ml and 88.5–95.9% of α-amantin recovery.

The application of removable nanoparticles as porogens has shown to be a very promising approach for creation of polymeric macroporous structure with enhanced accessibility of the analyte to the binding sites. A number of examples of the use for this purpose both organic and inorganic nanoparticles (NPs) can be found in recent literature. A molecularly imprinted film (MIF) has been designed for the quantitative determination of testosterone in urine with the polystyrene NPs [58]. Upon their removal a robust macroporous 3D matrix was formed, which allowed decreasing the limit of detection of testosterone up to 10^{-15} g/ml.

Molecular imprinted photonic hydrogels (MIPH) were prepared with silica nanoparticles to construct a novel colorimetric sensors for vanillin, ketamine and atropine detection [59,60]. In this case, a Bragg diffractive shift after the guest molecule binding was used in sensing platform to detect it with high selectivity and sensitivity. The photonic-sensitive colloidal crystals were formed by photo-polymerization under UV light in a presence of guest ligands with following silica nanoparticles etching by hydrofluoric acid. Constructed by this way smart chemosensors allowed cheap, handle and fast screening of the analyte in semi-quantitative manner (visible evaluation of the polymer color) in a wide range of concentrations.

3. RLIs in biosensor design

The specific ligand-receptor interaction and high affinity recognition of one molecule by another one have been successfully used at development and implementation in medicine of a huge number of ultrasensitive biosensors intended for the detection of biomarkers in complex biological fluids at early stages of various deceases [61–65]. Advancements in biosensor design allowed reaching the detection limit of several biomolecules at the *attomolar* level [66,67]. However, despite the intensive research, the development of reliable and highly sensitive quantitative assays and biosensors for efficient determination of low-abundance biomarkers still remains a key problem. The diagnostic devices need to meet several requirements, such as high selectivity and sensitivity, accuracy, reproducibility, long-term stability, fast response, low cost of fabrication, etc. Moreover, they should be user friendly and allow getting all necessary information in point-of-care manner without any discomfort for the patients. Typically, biosensors consist of three spatially connected units: (1) a detector, which receives a signal, (2) a transducer, and (3) an amplifier with signal processing system [68] (Fig. 4).

Recently the new types of reagentless biosensors, where the detection and signal-transducing unit were combined into one supramolecular domain, have gained attention allowing noticeable simplification of a whole system [69,70]. Various groups of biosensors can be classified, depending on the type of transducer, such as electrochemical [71], optical [72], microgravimetric [73], thermal [74], and magnetic [75], or biosensors, where a combination of various approaches is used for the conversion of biological signal to the electrical output.

A detecting unit is a fundamental part of biosensor, where high affinity biorecognition takes place. It defines the selectivity, sensitivity and stability of the biosensor [76]. Therefore, a big attention has been paid to the rational design and the development of this biosensing component and careful selection of the most efficient ligand-receptor pair. Depending on detector's recognition element, the biosensors can be classified as (1) catalytic or enzymatic type, where enzyme-substrate pair is used, and (2) affinity type (immunosensors, DNA sensors, etc.) with receptor-ligand interaction involving DNA or RNA, antibodies, aptamers, whole cells, synthetic or natural receptors [77,78].

From a general point of view, the specific analyte, which has to be detected, can be considered as a ligand, whereas a receptor, which is usually immobilized on a surface, plays a role of a capturing unit of the detector. Upon the formation of ligand-receptor affinity complex

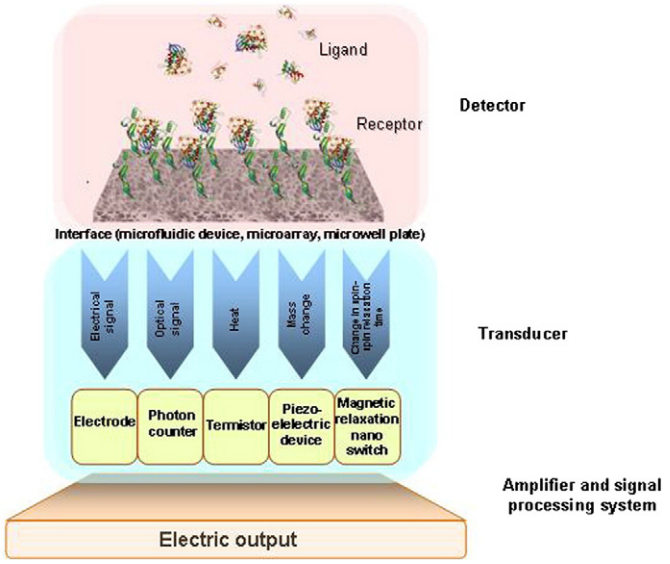

Fig. 4. A general scheme of a biosensor.

in the sensing domain, a change of the physicochemical properties of the detector takes place, which is converted and amplified by transducer. Myriads of biosensors aimed to detect and quantify various biomarkers have been described. Some of the most interesting and recent examples of them are gathered in Table 1.

The most known catalytic biosensors are based on the enzymes of oxidase type, such as horseradish peroxidase (HRP), glucose oxidase (GOD), uricase, the catalytic cycle of which includes easily detectable hydrogen peroxide. A disadvantage of the enzyme-based sensors is the dependence on many environmental factors, including pH, ionic strength, temperature, etc. Nevertheless, these ligand-receptor systems are often used in other types of biosensors for signal transduction and amplification. For example, fast simultaneous measurement of several cancer biomarkers was achieved at extremely low range of 5–50 fg/ml by using an electrochemical microfluidic chip [67]. In this case, a "sandwich" structure with the layers of gold and magnetic nanoparticles upon biomarker binding allowed their ultrasensitive electrochemical detection.

Recent advances in the surface plasmon resonance (SPR) technique, made it one of the most powerful analytical approaches for detection and monitoring of RLI events [102]. The modification of SPR-localized surface plasmon resonance (LSPR) combined with immunoassay has been shown as a promising label-free approach for fast detection of various biomolecules, for example, tumour marker α-fetoprotein (AFP) in human serum samples [79]. The biosensor, which was prepared by interference lithography and modified by covalently immobilizing AFP antibody on the surface of gold nano-mushroom arrays (GNMA), allowed highly sensitive detection of AFP with the limit of detection of 24 ng/ml.

Another detection method, based on electrochemiluminescence (ECL), has shown to be a powerful tool for the creation of highly sensitive and selective solid-phase and solution-phase RLI-based biosensors due to a number of advantages of ECL technique over many other analytical methods, such as absence of a background optical signal, precise control of reaction kinetics by controlling the applied potential, compatibility with solution-phase and thin-film formats and possibility to enhance its intensity with nanomaterials [103]. For example, an ultrasensitive and quantitative ECL assay for the determination of HIV biomarkers to monitor AIDS progression, as well as for the study of binding affinities and binding ratios of corresponding receptor-ligand complementary pairs, was developed by Xu et al. [88]. For this purpose, a high affinity interaction between T-lymphocytes' surface protein CD4 and glycoprotein gp120 of HIV-1 was used. This approach was also

Table 1
Examples of RLIs used for biosensor design.

Affinity pair	Detector	Disease	Sensitivity	Ref
Alpha-fetoprotein (AFP)/antibodies	Optical (surface plasmon resonance, SPR)	Testicular and liver cancer	24 ng/ml	[79]
Human thyroid stimulating hormone (hTSH), growth hormone (hGH), follicle stimulating hormone (hFSH), luteinizing hormone (hLH)/antibodies	Optical (SPR)	Hypothyroidism, acromegaly, reproduction and developmental disorders	1–6 ng/ml	[80]
Oxidized low density lipoprotein (oxLDL)/CD36 receptor	Optical (SPR)	Atherosclerosis	–	[81]
Vascular endothelial growth factor receptor (sVEGFR-1)/VEGF	Optical (SPR)	Myelodysplastic syndrome, acute myeloid leukemia	25 ng/ml	[82]
Uric acid/uricase	Optical (Fluorescence)	Uric acid disorders, leukemia and pneumonia	125 nmol/ml	[83]
Folate receptor/folic acid	Optical (Fluorescence)	Various types of cancer and inflammatory diseases	30 pM	[84]
SNAP-25 peptide/Botulinum neurotoxin serotype A light chain (BoNT-LcA)	Optical (fluorescence resonance energy transfer, FRET)	Botulism	1 fg/ml	[85]
Prostate specific antigene (PSA), Interleukin 6 (IL-6)/antibodies	Optical (electrochemiluminescence, ECL)	Prostate cancer	0.25–1.00 pg/ml; 4.20 fg/ml	[86,87]
HIV envelope protein gp120/CD4/antibody	Optical (ECL)	AIDS	16 fmol/ml	[88]
Apurinic/apyrimidinic endonuclease 1 (APE-1)/antibody	Optical (ECL)	Various types of cancer	0.3 fg/ml	[66]
Cardiac troponin I (cTnI)/antibody	Optical (ECL)	Myocardial injury	0.083 pg/ml	[89]
8-Hydroxy-2′-deoxyguanosine (8-OHdG)/antibody	Optical (ECL)	Various types of cancer, diabetes, neurological disorders	0.085 ng/ml	[90]
Carbohydrate antigen 15-3 (CA15-3)/antibody	Optical (ECL)	Metastatic breast cancer	0.033 μg/ml	[91]
Vascular endothelial growth factor (VEGF)/antibody	Optical (chemiluminescence)	Various types of cancer	60 pg/ml	[92]
Thyroid hormone receptor (TRβ) mutants/various agonists	Optical (colorimetry)	Resistance to thyroid hormone, weight loss, hair loss, short stature	–	[93]
Ferritin/antibody	Microgravimetric (quartz crystal microbalance, QCM)	Various types of cancer	0.1 ng/ml	[94]
Folate receptor/folic acid	Microgravimetric (QCM)	Various types of cancer	430 cells/ml	[95]
Bacterial lipopolysaccharides (LPS)/anti-LPS peptides	Microgravimetric (QCM)	Bacterial endotoxin detection	–	[96]
Human epidermal growth factor receptor (HER-3)/antibody	Electrochemical (impedance, voltammetry)	Various types of cancer	4 pg/ml	[97]
Epidermal growth factor receptor (EGFR)/GE11 peptide	Electrochemical (impedance)	Various types of cancer	0.037 pg/ml	[98]
Dengue virus/C-type lectin domain family 5 receptor	Electrochemical (impedance)	Dengue fever	9.5×10^5 pfu/ml	[99]
IL-6, IL-8,VEGF, VEGF-C/antibodies	Electrochemical (amperometry)	Various types of cancer	5–50 fg/ml	[67]
Immunoglobulin A (IgA)/antibody	Electrochemical (impedance)	Immunodefficiency	0.01 ng/ml	[100]
Glucose/glucose oxidase	Electrochemical (impedance, voltammetry)	Various diseases	35 nmol/ml	[101]
Cholera toxin B (CTB)/ganglioside GM1	Magnetic	Cholera	40 pM	[75]

successfully applied to determine the binding affinity of CD4 with monoclonal antibody (mAB), aiming the identification of potential neutralizing agents for HIV treatment to block the interaction of gp120 with CD4 and to prevent host T-cells infection by HIV. CD4 was conjugated with $[Ru(bpy)_3]^{2+}$-NHS ester to prepare CD4-tag via a ruthenylation reaction. Electroluminescence of $[Ru(bpy)_3]^{2+}$ in a presence of TPA allowed quantitative determination of CD4 content in a wide concentration range (0–2 nM) in solution with a high sensitivity (16 fmol). Moreover, it was shown that mAB could potentially neutralize the HIV infection of T-cells by blocking the binding of gp120 to CD4.

The different approach was used for the construction of ultrasensitive solid-state ECL biosensor to determine apurinic/apyrimidinic endonuclease 1 (APE-1), a multifunctional gene regulatory protein, which can serve as a biomarker in cancer [66]. In this case, an enhancement

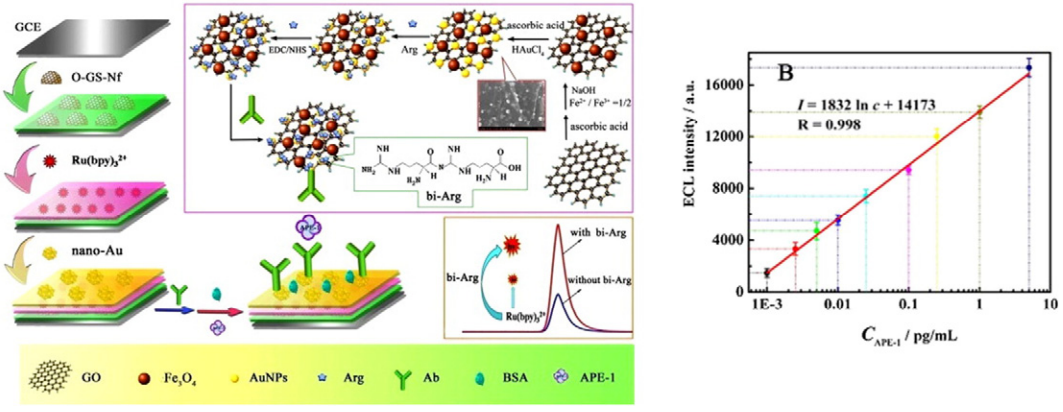

Fig. 5. Schematic illustration of solid-state ECL immunosensor preparation process based on the new $Ru(bpy)_3^{2+}$/bi-Arg system (left) and ECL responses of the immunosensor to different concentration of APE-1 (right) [66].

of ECL signal was achieved by using bi-arginine as co-reactant for $[Ru(bpy)_3]^{2+}$-based detection system (Fig. 5). Graphene oxide (GO) with Ru^{2+} complex and onion-like mesoporous graphene sheets (O-GS) modified with bi-arginine were used to construct two main biosensor layers containing antibodies against APE-1. The interaction between these layers upon their linking by APE-1 induced strong electrochemiluminescence with linear dependence on the amount of biomarker at extremely low level in the range of 1.0 fg/ml–5.0 pg/ml.

RLI-based biosensors and accurate quantitative real-time determination of biomolecules as biomarkers of various metabolic processes in cells, as well as the quantification of the cells without affecting cell viability, have an enormous potential in tissue engineering and regenerative medicine [104,105]. This approach is especially important for the control of the concentrations in the supporting liquids of glucose as a marker of the cell activity, hydrogen peroxide as a marker of oxidative stress and hypoxic conditions, and various cell signaling molecules, such as growth factors, metalloproteinases, ATP, ADP, etc.

4. RLIs in biomaterials design

A property of cells to respond to extracellular stimuli triggered by ligand-receptor interactions have been widely used for the construction of smart biomaterials with a wide range of physicochemical and biomedical applications in biotechnology and pharmacology, tissue engineering, etc. Although the biomaterial structure can also favour the desired direction of biosystem development [106], the presence of low molecular weight or/and macromolecular (proteins) ligands, which can regulate downstream signaling events, is a crucial factor for biomaterial fabrication. The various approaches have been developed to introduce the components of ligand-receptor pairs into the structure of such artificial matrices mimicking extracellular environment. The construction of various signaling ligand-receptor pairs into cell/biomaterial systems can be achieved by different ways, including (a) modification of biomaterial with ligands (both immobilized and in soluble form), enabling to bind to cell receptor to provide the desired cell signals, and (b) the use of co-culture system-based biomaterials, where the interaction between different types of cells occurs with reciprocal signaling pathways activation (Fig. 6).

This is probably the most complicated case of RLI-based bionanotechnological field, where the created bioactive materials are intended to be used in the living systems with their complex signaling pathway network, which can lead to undesired side effects or completely change the biological response. Therefore, along with the choice of the ligand-receptor pair, a number of other factors have to be taken into consideration while designing biomaterials to be incorporated in living organism, such as biomaterial structure and the presence of adjacent cells (co-culture systems).

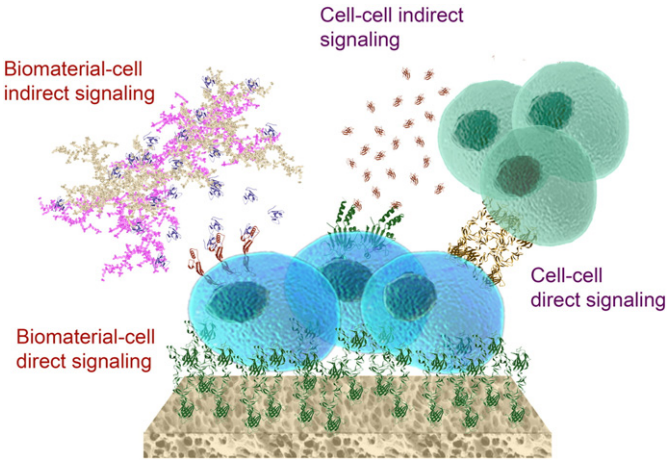

Fig. 6. Structure of cell-biomaterial system with various RLI-based signaling pathways.

Along with soluble and fixed cell signaling factors of extracellular matrix (ECM) and cell-cell interactions, the contact surface between cell and artificial support or matrix usually is involved in cell homeostasis regulation and cell signaling pathways. Cell adhesion to the extracellular artificial matrix, as well as the fate of the cell, are strongly dependent on the structure of the support and its physicochemical properties, such as charge, hydrophobicity, morphology and mechanical properties [107]. A number of excellent reviews are devoted to the biomaterial characteristics necessary for different biomedical applications regarding to tissue engineering and repair [108–110]. The ideal material for cell support in tissue engineering and regenerative medicine should act as artificial extracellular matrix and protect the cell from external mechanical stress, be biocompatible and promote cell adhesion, proliferation and, if necessary, differentiation with the aim of different extracellular physical, biological and chemical cues. In the case of three-dimensional structure, it has to allow vascularisation of new forming tissue, free access of nutrients and the removal of metabolic products. The temporary support has to be degraded to non-toxic products within the time necessary for the formation of self-supporting matrix. The numerous biomimetic materials of different type have been applied in tissue engineering and tissue repair. These biosupports can be divided into biomaterials originated from metals, such as stainless steel or titanium [111], ceramics and glasses, hydroxyapatite, tricalcium phosphate, calcium carbonate, zirconia [112,113], polymers of various types [114–116], inorganic-organic hybrids [117] and have various morphology, ranging from nanoparticles to bulk materials. Surface modification of cell supporting matrix can be based both on non-covalent interaction between biomolecules and support, such as electrostatic or van der Waals forces [118], or covalent immobilization [109,119], depending on the final application of designed material and desired biological response [120]. It can include the enhancement of cell adhesion or its prevention and cell detachment, cell migration to the desired location, increase of the time of the signaling event and ligand-sustained release for cell proliferation and differentiation.

The growing interest to co-culture constructs is explained by better fitting to the natural tissues, where different cell types are co-existing and interacting with each other, though it is not very easy to regulate all the processes taking place in such complex systems. There are many parameters and multiple interactions, which have to be taken into account when working in cell multi-cultures, such as cell ratio, cell number, contribution of different cell types in cell proliferation and differentiation [121]. Nevertheless, co-culture systems have been used in many approaches to tissue engineering and regenerative medicine, such as promotion of osteogenesis, vascularisation, stem cells differentiation etc. For example, a cross-talk between human and rat long bone osteoblast-like cells (HOB and LOB) and HUVEC with paracrine VEGF stimulation, mediated by endothelial cells was observed during ontogenesis [122]. VEGF-mediated paracrine signaling between endothelial cells and mural cells with increased junction formational complexes formation and increased resistance of endothelial cells to apoptosis was also observed [123]. Co-cultured with HOBs and mesenchymal stem cells HUVECs stimulated their proliferation and this effect was more pronounced in direct co-cultures, rather than in indirect ones, probably due to the contacts between cell membranes and direct mechanism of signaling transduction [124]. Cells migration was induced by *co*-culturing of human vascular smooth muscle cells (hVSMCs) with wound-healing phenotype monocytes into porous polar hydrophobic-ionic polyurethane. It was shown, that hVSMCs alone remained at the perimeter of the scaffold, whereas in co-cultures system they are translocated into the pores of matrix. Moreover, the increase of cells attachment and their viability was observed [125]. Biomaterials of different structure for bone tissue engineering were used as scaffolds for co-cultured cells to investigate the process of vascularisation with human dermal microvascular endothelial cells (HDMEC). It was shown that HDMEC cells alone do not form microcapillary-like structures, whereas the addition to the biosystem of HOS or osteoblast-like cells MG-63

promoted a tissue-like self-assembly of cells with a strong PECAM-1 expression at the cell surfaces and pre-vascularization. Interestingly, the addition of exogenous angiogenic stimuli did not affect the microcapillary-like structure formation [126]. More complex multi-cell culture system was designed by three-dimensional printing and layer-by-layer technique in a solid gel composed of sodium alginate-collagen and calcium chloride. The bioprinted constructs, containing human amniotic fluid-derived stem cells, canina smooth muscle cells and bovine aortic endothelial cells, maintained cell survival, proliferation and differentiation to form functional vascularised tissue [127].

4.1. Receptor-ligand interactions in direct signaling

Direct signaling mechanism, which is mediated by receptors and ligands located on adjacent surfaces, is widely used in different biomimetic approaches for the cells attachment to natural or artificial surfaces or matrices. For this purpose, various ligands, receptors and macromolecules found in extracellular matrix and plasma membrane can be incorporated into biomaterials.

4.1.1. Immobilization and modification of adhesive ligands

There are many macromolecules, such as matrix proteins (elastin, collagen, fibronectin, vitronectin), glycosaminoglicans, glycoproteins, which act not only as a cell support and cell adhesion molecules (CAM), but also participate in signal transduction and are involved in cell proliferation, growth, differentiation and mobility [128,129]. A number of ligands recognized by these biopolymers have been used for the biomaterials fabrication in order to induce various effects. The primary cell plasma membrane receptors, which are involved in cell adhesion to the extracellular matrix and signal transduction, are integrins. They recognize certain small peptide sequences, such as REDV [130], IKVAV [131], YIGSR [132], PHSRN [133], and RGD [134], which have been routinely used in different tissue engineering approaches and regenerative medicine [135].

The presence of RGD-containing molecules in the scaffold structure promotes cells adhesion to the surfaces of various types, as well as their migration and proliferation. For example, RGD-containing ligand immobilized on poly(ε-caprolactone) (PCL) not only enhanced fibroblasts adhesion and well-spreading on the solid support, but also changed their morphology and induced the formation of filopodia [136] (Fig. 7, I).

The opposite effect (detachment from the biomaterial surface) can be achieved, when necessary, by the shielding of RGD ligands, as, for example, in the case of construction of multi-domain structures with two complementary leucine zippers, which enable heterodimer formation [137]. The shielding of adhesion surface was achieved by covalent modification by RGD motif with one of leucine zipper domains, whereas the second one was attached to PEG polymer in a solution. The heterodimer formation led to covering of the surface with PEG and inaccessibility of RGD ligand to the cellular integrin receptors. Deshielding of the support with following cell adhesion took place after addition of excess of first leucine zipper molecule. When the excess of second leucine zipper was added to the cells already attached to the support, cells detachment was observed.

Often it is necessary to block cell adhesion, in order to reduce inflammatory response and to prevent tissue rejection in different transplantation approaches. This is particularly important at the design of biomaterials contacting with cardiovascular system. For example, the blockage of an adhesion mediated by ICAM-1, VCAM-1, VLA-5 with monoclonal antibodies could significantly improve the results of the transplantation [138]. The easiest way to design an inert biomaterial is to cover the surface ligands or receptors with immunosuppressive coatings, for example, polyethylene glycol. Immunological camouflage with the aim of methoxypoly(ethylene glycol) grafting to allogeneic lymphocytes showed effective immunosuppression and thus can have a potential for donor tissue engraftment [139]. The same approach was used to hide antigenic determinants of red blood cells [140].

When using RLI in the design of biomaterials for tissue engineering and repair, it is important also to take into consideration the effect of ligand density and patterning on support surface [141]. The immobilized ligand gradients can govern cell adhesion that was demonstrated with RGD-modified gold nanoparticles. Particle spacing gradients with the range 50–250 nm allowed determination the minimal gradient strength necessary for sensing by the cell. It was found, that cells could feel the

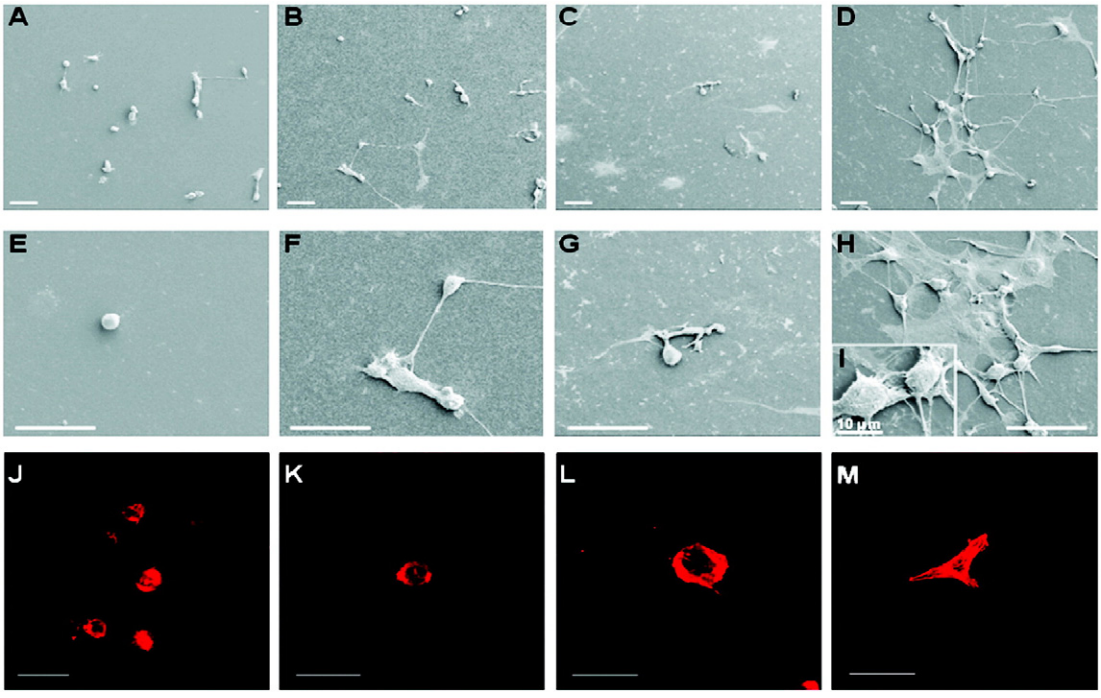

Fig. 7. Scanning electron microscope micrographs (A and E - PCL; B and F - PCL-NH_2; C and G - PCL-GA-GYDGR; D, H, and I - PCL-GAGRGDY). Confocal laser scanning microscope images of phalloidin staining the microfilaments (J - PCL; K - PCL-NH_2; L - PCL-GA-GYDGR; and M - PCL-GA-GRGDY). The scale bar is 50 μm [136].

spatial variations with the distance of <1 nm between RGD molecules. Ligand patch spacing strongly influenced the cell morphology from radial, well-spread adherent cells at about 50 nm to elongated distant cells at 80 nm [142]. Moreover, the RGD ligand nanospacing can promote cell differentiation. It was shown, that increased RGD-RGD distance attenuates cell adhesion and spreading of mesenchymal stem cells, but was beneficial for osteogenesis and adipogenesis [143]. As it was shown for dendrimer-bound RGD, nanoclustering of ligand, even in a solution, can noticeably improve cell adhesion [144]. The local distribution of RGD motifs on the surface influences both cell adhesion and motility. The dependence of cell migration speed was observed for clustered YGRGD peptides. The clustering of ligand significantly reduced its density necessary for cell migration [145].

The cleavable RGD motif was shown not only to promote cell adhesion and survival, but also induce cell differentiation in PEG hydrogels. The specific matrix metalloprotease (MMP-13) cleavable linker was used to mimic native differentiation timeline of encapsulated human mesenchymal stem cells and to adjust the time of release of RGD containing molecule in order to enhance hondrogenesis [146]. The cleavable metalloproteinase-sensitive RGD-containing ligand was used to construct the scaffold based on PEG-heparin matrix for the primary endothelial cells encapsulation. It was shown that a gradual degradation of the ligand and reorganization of biomaterial impaired the cell spreading, viability and three-dimensional cell growth [147].

The presentation of different ligands and their concentration can induce different effects on cell behaviour. They can both enhance and suppress the action of each other. For example, the introduction into biomaterial structure of fibronectin-derived peptide PHSRN along with RGD strongly influenced the normal human osteoblasts differentiation. It was shown that they have synergic effect and enhanced differentiation in alginate gel containing >33% of RGD. The presence of these two ligands also enhanced the calcium deposition, which depended on their ratio and relative distribution [148]. In the case of EGF and RGD, the growth factor can act either as an adhesion enhancer or its suppressor, and stimulate cell growth, depending on its concentration, RGD density, duration of action or the form of application (immobilized or soluble forms) [149]. The ligand density is particularly important when working with the stem cells, which can change their fate depending on the structure and amount of the ligand on the surface of supporting matrix that was observed for the case of cyclic and linear RGD peptides (Fig. 8) [150].

The level of alkaline phosphatase (a marker for osteogenesis), as well as the expression runt-related transcriptional factor 2 (Runtx2), specific for osteoblasts, was noticeably higher for higher concentration of high affinity cyclic ligand. On the contrary, in the case of linear RGD peptide, the lower affinity and density on a gold support caused the differentiation to neuron-like cells, whereas the high density of this ligand led to the expression of miogenic marker genes.

The receptor clustering, along with the orientation and gradient of adhesive ligands, can also be involved in RLI. As it was shown for TGF-β, such effect can be used for a spatial control of cell attachment to the support and signal transduction [151]. TGF-β takes place in different cell regulation pathways and its interaction with TβRI and TβRII receptors is initiated by the receptor oligomerization. It was shown that

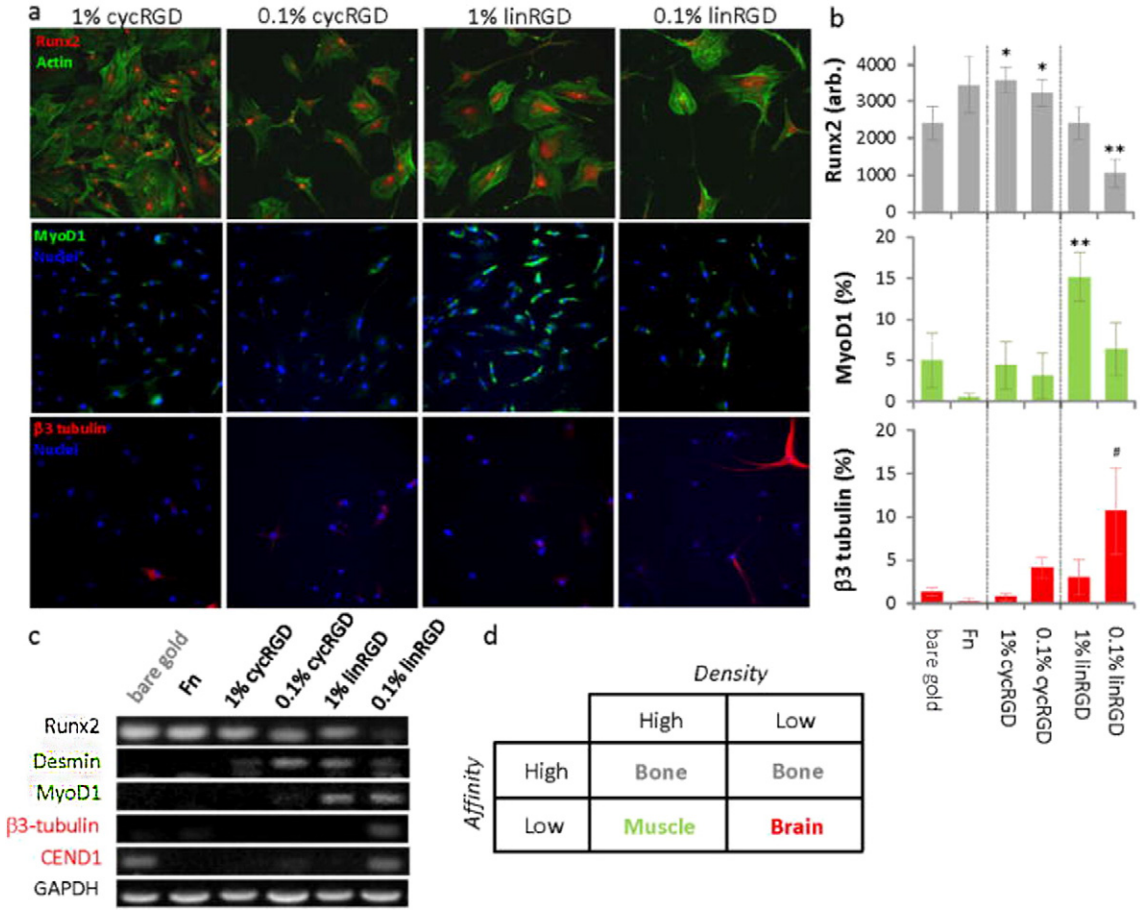

Fig. 8. The density and affinity of adhesion ligand influence the differentiation of mesenchymal stem cells. Cells were cultured on monolayers presenting either the cyclic (high affinity) or linear RGD (low affinity) peptide at high (1%) or low (0.1%) density and differentiation was analyzed using (a, b) immunofluorescence imaging of markers for osteogenesis (Runx2), myogenesis (MyoD1) and neurogenesis (b3-tubulin), and (c) expression analysis by RT-PCR of lineage specific transcripts; (d) the Table summarizing the preferred differentiation outcome for cells cultured on the four monolayer surfaces. Scale bar is 20 μm [150].

sensibilization of these receptors by specific peptides promoting cell-surface contact led to potentiating and amplification of TGF-β signaling followed by the epithelial-mesenchymal transition.

4.1.2. Immobilization of growth factors

Unlike adhesion ligands, the function of many growth factors and morphogenic proteins requires cell internalization upon binding and action in indirect way *via* paracrine or autocrine mechanisms [152]. Nevertheless, their signaling pathways very often are bound to cell adhesion and their covalent immobilization on a solid matrix in many cases can promote both cell spreading and survival more strongly than the same soluble molecules [153]. For example, covalently bound to a glass surfaces or to PEG hydrogel TGF-β strongly increased matrix production with vascular smooth muscle cells even in the absence of adhesive ligands and better than with the same concentration of soluble growth factor [154]. A tethering of brain-derived neurotropic factor (BNDF) onto poly(ε-caprolacton) nanofibers had stronger effect on proliferation and differentiation of neural stem cells toward neuronal and oligodendrocyte specification than its soluble form [155]. A macroporous scaffold on the base of Sponceram® (zirconium dioxide ceramic) covered with 2-deoxy-N-methacrylamido-D-glucose was designed for immobilization of MC3T3 and human mesenchymal stem cells. Covalent modification of polymer surface with bone morphogenic protein (BMP-2) to promote cell differentiation demonstrated to be effective for the osteogenesis induction [32]. Encapsulation of bone morphogenetic proteins BMP-2 and BMP-14 into thermoresponsive elastin-like nanoparticles was used for sustained release of these growth factors for 14 days. Effective delivery with synergic effect and increased bioactivity were observed when using both signaling molecules [156] (Fig. 9).

In other cases, the prevention of the internalization of ligand-receptor pair by the immobilization of one of the components can lead to the blocking of one of signaling pathways, if the ligand promotes different effects, or even blocks completely its action. It can happen, when ligand-receptor pair initiates the signals from different locations within the cell, for example, activated by NGF TRK receptors [157]. Cell survival response triggered by NGF and initiating on plasma membrane was increased in the case of covalent attachment of NGF to a solid support, when internalization of ligand-receptor pair is not required, whereas the neuronal differentiation promoted within endosomes was strongly decreased [158].

A creation of growth factors gradients, as well as the presence of different immobilized growth factors, showed to change signalling pathways and the activity of signalling, as in the case of nerve growth factor (NGF) and neurotrophin-3 (NT-3) co-localized in poly(2-hydroxyethylmethacrylate) and poly(L-lysine) matrices, where a cooperative function of these two factors was observed [159].

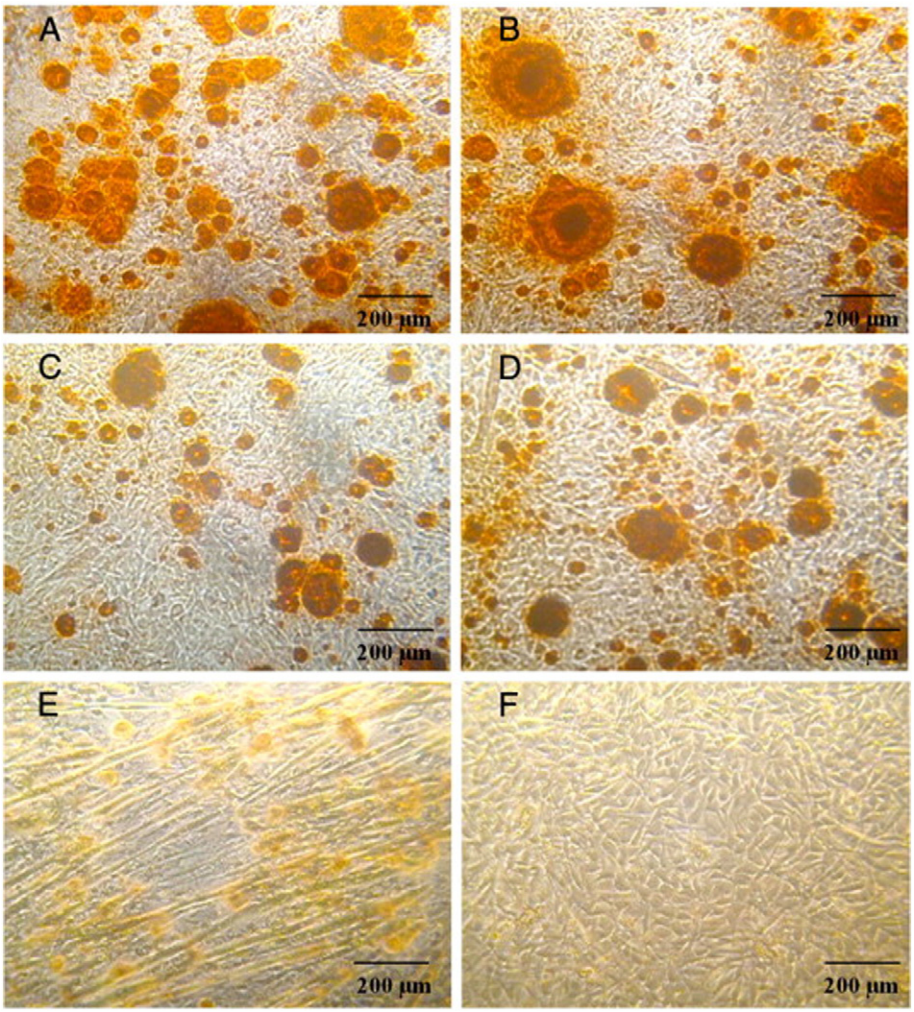

Fig. 9. Alizarin Red mineralization staining of showing sites of calcium-phosphate deposits (orange colour) in C2C12 cell line differentiated into osteoblast after 14 days of culture with particles loaded with BMP-2 (A), BMP-2 and BMP-14 (B), and the positive controls of BMP-2 (C) and BMP-2 + BMP-14 (D) added to culture media, at 2.5 μg/ml. Unloaded particles did not showed any mineralization (E). Unstained cells treated with particles loaded with BMP-2 + BMP-14 showed osteoblast morphology (F) [156].

4.1.3. Immobilization of other ligands

Another interesting approach to induce direct cell signals in biomaterial design is the immobilisation of Delta/Jagged ligands on the cell-biomaterial surface contact or co-culture of Delta and Jagged ligand-containing cells in order to activate Notch signaling cascade, which is known to be crucial for the determination of hematopoietic stem cells (HSCs) fate and T-cells development. In this case, it was shown the importance of a proper orientation of ligand-receptor pair [160]. Recombinant chimeric protein Jagged-1-Fc, modified with Fc-domain of immunoglobulin G, was immobilized on aminated glass substrate using orienting protein A and was used for the induction of proliferation and differentiation of HSCs. A comparison of the effect on cell proliferation between oriented (immobilized) and randomly distributed (coated) ligands showed for second case the absence of cell lines derived from HSC and no Notch signaling.

Jagged-1 modified poly(2-hydroxyethyl methacrylate) was shown to promote tight clustering and differentiation of human-derived keratinocytes and appeared to be sensitive to cell density. It was found also, that affinity-bound ligand was more effective than directly bound one, probably, due to the proper orientation toward the Notch receptor, as well as due to the inherent clustering of the ligand on the antibody used for the immobilization [161]. T-cells development from mouse bone marrow hematopoietic stem cells was observed, when Notch ligands were covalently immobilized or absorbed on the solid support [162].

A layer-by-layer molecular assembly approach was used for the attachment of DL-1 Notch ligands into porous polyacrylamide hydrogel scaffold with subsequent human stem cells immobilization. In this case, the effective cell differentiation was also observed [163]. More complex cell support with spherical pores (inverted colloidal crystal, ICC), forming highly organized 3D structure, was designed to mimic the thymic tissue [164]. The same layer-by layer technique was applied for coating of a scaffold surface with several layers of clay and poly(diallyldimethyl ammonium chloride) with following deposition of DL-1. It was found, that the artificial 3D-matrix not only efficiently supported the adhesion of mononuclear and CD34+ cells, but also induced the development of pre-erythrocytes and T-cell differentiation. On the contrary, the "naked" scaffold could not promote cell attachment and most pores were empty (Fig. 10).

Eph/ephrin ligand-receptor pair has also been used for the preparation of biomimetic scaffolds, though not so often as the other ligand-receptor pairs involved in juxtacrine direct signaling. For example,

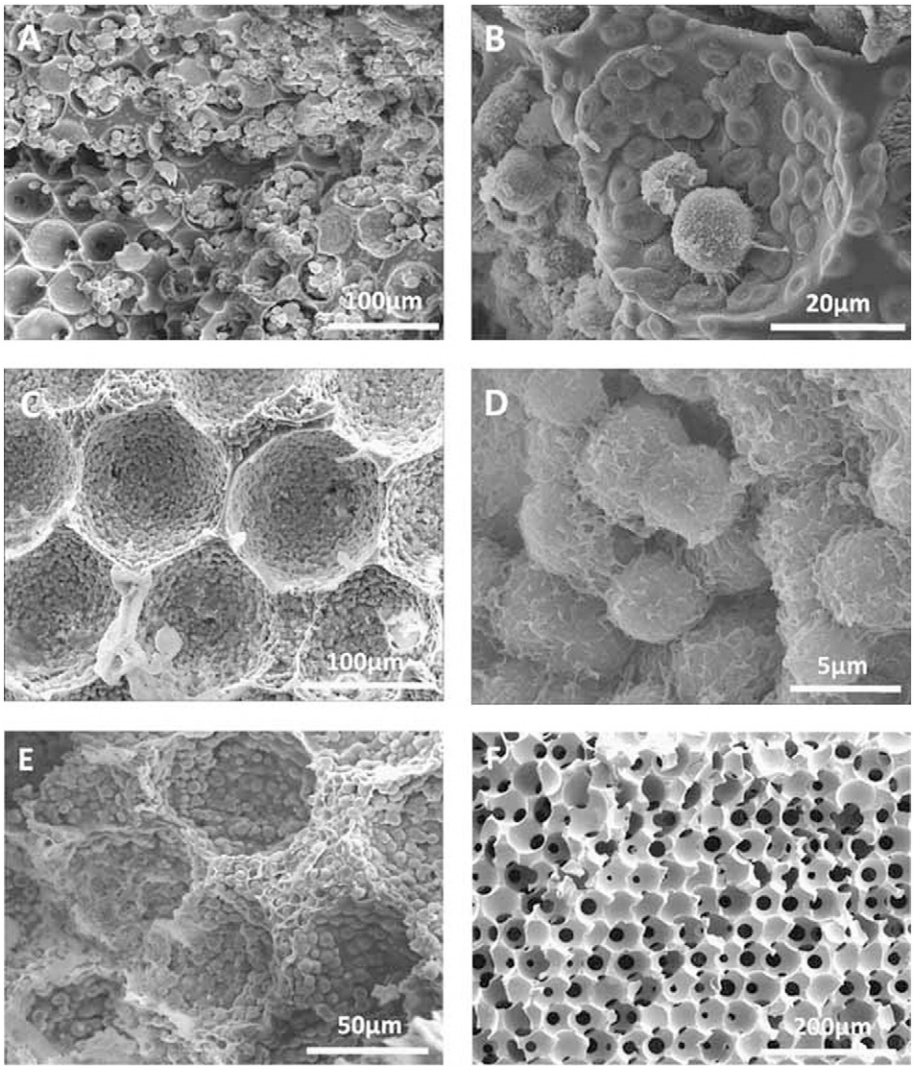

Fig. 10. SEM images after 15 days of culture. Mononuclear cells (A, B). (A) Various cell types coexist deep inside pores. (B) Development of pre-erythrocyte and migrating dendritic-like cells across pores. (C, D) Isolated CD34+ cells from bone marrow-derived mononuclear cells. (C) The DL-1 Notch-ligand immobilized pore surface densely covered with cells. (D) Due to similar morphology and size, it is hard to distinguish developing T-lymphocytes from CD34+ HSCs under SEM. (E, F) Control experiments of 15-day-cultured CD34+ cells. (E) ICC scaffolds could support cell adhesion without a DL-1 Notch ligand coating. (F) Without a clay/PDDA LBL coating (bare hydrogel), ICC scaffolds could not allow any cell adhesion [164].

immobilized in poly(ethylene glycol)-diacrylate hydrogel ephrin-A1 ligand induced cell adhesion and endothelial tubule formation in endothelial cell culture as early stage of angiogenic process [165].

4.2. Indirect signaling in tissue engineering and repair

Indirect signaling, which is mediated by the flux of extracellular molecules or vesicles, can be applied in tissue engineering and repair by introduction of signaling molecules, such as growth factors (EGF, VEGF, FGF), bone morphogenetic proteins, cytokines and chemokines, into extracellular matrix. These molecules have very short half-life in biological liquids and are rapidly degraded *in vivo*. Therefore, different approaches and delivery systems have been developed to induce indirect signals activated by these proteins to increase their longevity.

Along with the addition of these molecules to the designed biosystems in soluble form, they can be incorporated into a biomimetic scaffold with their temporal and spatial release and triggering or modulation of tissue formation, cell differentiation, adhesion or mobility. Passive release of signaling molecules can be achieved by their encapsulation in the matrix by simple adsorption, by complexation or by using of hydrolysable or pH-dependent covalent binding to the support. Permanent covalent immobilization of ligands or receptors, which are involved in the indirect mechanism of signal transduction, can increase their retention time and time of life due to the prevention of endocytosis and enzymatic degradation. It can form by that their local store, which is a doubtless advantage, when it is necessary to achieve their continuous action. Nevertheless, covalent immobilization can lead to the activation of other signaling pathways. For example, the comparison of the effects caused by vascular endothelial growth factor covalently bound to the matrix in bioactive orientation and the same protein in a solution triggered phosphorylation of VEGFR-2 receptor, but the HUVEC cells had different morphologies in a presence of immobilized or soluble forms of VEGF [166]. Therefore very often it is preferable to have the biomaterials that are able to release signaling molecules for their correct functioning via diffusion or matrix degradation. Cell signaling molecules have been immobilized in many biomimetic hydrogels based on different polymers, such as hyaluronic acid, chitosan, gelatine, as well as copolymer of lactide and glycolid (PLGA) or PEG hydrogels [167,168].

Methylcellulose was used for the tuneable sustained release of SH3-rhFGF2 fusion protein from hyaluronic hydrogel modified with SH3-binding peptide [169]. The similar approach was used for PEG hydrogel preparation to immobilize the affinity pair peptide/basic fibroblast growth factor with the slow release of FGF-β using different architecture of affinity peptide, its valences and the ratio between peptide and protein in order to achieve the desired retention of growth factor in the matrix [170]. Keratinocyte growth factor (KGF) was fused with elastin-like peptide to form self-assembled nanoparticles, which strongly enhanced keratinocyte and fibroblast proliferation in re-epithelialization and wound healing [171].

Another approach to achieve the release of angiogenesis-related factors, such as VEGF, PDGF, FGF-β, IL-8, was performed by using the innate capacity of peripheral blood cells to produce angiogenic factors under hypoxia conditions for the design of cell-matrix depots for angiogenic therapy [172]. A promising method for delivering of multiple factors in a time-dependent manner is a construction of multi-layer scaffolds, where the inner layers release the signaling molecules with a delay depending on matrix degradation rate or on the time, necessary for the signaling factor to reach the surface of the matrix. For example, such approach was used to reach time-dependent release of TGF-β3 and BMP-2 from alginate hydrogel in order to induce differentiation of mesenchymal stem cells. It was shown, that contemporary release of both signaling factors was needed for bone tissue formation [173]. The same synergic effect on osteogenesis was observed, when BMP-2 and Wnt1 were incorporated into gelatine/β-TCP matrix [174]. A biodegradable scaffold composed of poly(caprolactone)/β-tricalcium phosphate/growth factor/poly(acrylic acid) multi-layers with BMP-2 and VEGF in different ratios was designed to induce differentiation of HUVEC cells. The sustained release of BMP-2 was observed, whereas VEGF was eluted from the matrix first and promoted increased vascularisation [175].

The similar approach of time-dependent delivery of a combination of growth factors was used in [176], where PLA/alginate composite with VEGF and BMP-2 was prepared to induce osteogenesis of HMBSC cells and showed the increase of quantity of regenerated bone tissue.

A bioscaffold on the base of poly(2-hydroxyethylmethacrylate) microporous gel with a gradient of the nerve growth factor (NGF) was designed for the neurite guidance [159]. It was demonstrated that soluble and immobilized forms of NGF were able to promote neurite extension, but in case of immobilized form of the growth factor the PC12 cell neuritis were guided up the gradient.

5. Conclusions

RLIs are the crucial part of the majority of all the biological processes and form an extremely complex network of interacting molecules, which regulate homeostasis through a variety of cell signaling pathways. In the past decades high throughput proteomics and genomics technologies, as well as computational modelling and simulation, have generated an enormous amount of data on the details of the interaction between ligands and receptors, which were used in a countless RLI-based approaches in bioanalytics, bioinformatics, nanotechnology, nanomedicine, industrial biotechnology, drug design, etc. In this review, we tried to summarize various most representative and recent examples of ligand-receptor interactions, which lie on the base of several bionanotecnological applications. In particular, some novel approaches for the preparation of monolithic sorbents and molecularly imprinted polymers bearing artificial ligand or receptor for highly selective recognition of counter pair have shown to be very effective and promising for separation of complex biological mixtures and purification of low-abundant biomolecules, as well as for the design of highly sensitive biosensors. Another cutting-edge field of biomedical research is the design of smart (biomimetic) materials for regenerative medicine and tissue engineering, where ligand-receptor interactions are used for direction of the cell development in a specific manner. A number of approaches can be found in the recent literature describing the optimization of the biomaterial structure in order to induce a desired cellular response. Noticeable progress has been achieved in this field. Nevertheless, it is still remain challenging to identify which ligands are bound by a given receptor, to reveal the details of their interaction or to understand the place of ligand-receptor pair in a complex network of biomolecules. Thus, this review was aimed not only to highlight some the most important results of RLI applications, but also to underline the importance of continuous investigation in this field.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.msec.2016.07.072.

References

[1] P.M. Dean, Molecular Foundations of Drug-Receptor Interaction, Cambridge University Press, Cambridge, 1987.
[2] K. Hollenstein, J. Kean, A. Bortolato, R.K.Y. Cheng, A.S. Doré, A. Jazayeri, R.M. Cooke, M. Weir, F.H. Marshall, Nature 499 (2013) 438–443.
[3] B. Zuber, N. Unwin, Proc. Natl. Acad. USA 110 (2013) 10622–10627.
[4] T.P. Garrett, N.M. McKern, M. Lou, T.C. Elleman, T.E. Adams, G.O. Lovrecz, H.J. Zhu, F. Walker, M.J. Frenkel, P.A. Hoyne, R.N. Jorissen, E.C. Nice, A.W. Burgess, C.W. Ward, Cell 110 (2002) 763–773.

[5] G.H. Park, T. Morizumi, Y. Li, J.E. Hong, E.F. Pai, K.P. Hofmann, H.-W. Choe, O.P. Ernst, Angew. Chem. Int. Ed. 52 (2012) 11021–11024.
[6] T. Kenakin, M. Williams, Biochem. Pharmacol. 87 (2014) 64–77.
[7] P. Imming, C. Sinning, A. Meyer, Nat. Rev. Drug Discov. 5 (2006) 821–834.
[8] H. Lodish, A. Berk, S.L. Zipursky, P. Matsudaira, D. Baltimore, J. Darnel, Molecular Cell Biology, fifth ed. W. H. Freeman and Company, New York, 2000.
[9] M. Petukh, S. Stefl, E. Alexov, Curr. Pharm. Des. 19 (2013) 4182–41890.
[10] D. Vance, M. Shah, A. Joshi, R.S. Kane, Biotechnol. Bioeng. 101 (2008) 429–434.
[11] L.L. Kiessling, J.E. Gestwicki, L.E. Strong, Curr. Opin. Chem. Biol. 4 (2000) 696–703.
[12] S. Endo, S. Betsuyaku, H. Fukuda, Curr. Opin. Plant Biol. 21 (2014) 140–146.
[13] S.Z. Grinter, X. Zou, Molecules 19 (2014) 10150–10176.
[14] C.A. Helm, W. Knoll, J.N. Israelashvili, Proc. Natl. Acad. Sci. USA 88 (1991) 8169–8173.
[15] C.L. Hung, C.C. Chen, Drug Dev. Res. 75 (2014) 412–418.
[16] D.S. Hage, J.A. Anguizola, C. Bi, R. Li, R. Matsuda, E. Papastavros, E. Pfaunmiller, J. Varga, X. Zheng, J. Pharm. Biomed. Analysis 69 (2012) 93–105.
[17] N.E. Labrou, J. Chromatogr. B 790 (2003) 67–78.
[18] X. Fang, W.W. Zhang, J. Proteome 71 (2008) 284–303.
[19] E.G. Vlakh, V.A. Korzhikov, T.B. Tennikova, Russ. Chem. Bull. Int. Ed. 61 (2012) 937–961.
[20] A. Podgornik, S. Yamamoto, M. Peterka, N.L. Krajnc, J. Chromatogr. B 927 (2013) 80–89.
[21] F. Svec, T. Tennikova, Z. Deyl, Monolithic Materials: Preparation, Properties and Applications, Elsevier, Amsterdam, 2003.
[22] N.D. Ostryanina, G.P. Vlasov, T.B. Tennikova, J. Chromatogr. A 949 (2002) 163–172.
[23] E. Calleri, S. Ambrosini, C. Temporini, G. Massolini, J. Pharm. Biomed. Anal. 69 (2012) 64–76.
[24] T. Nema, E.C.Y. Chan, P.C. Ho, J. Pharm. Biomed. Anal. 87 (2014) 130–141.
[25] F. Yang, J. Mao, X.W. He, L.X. Chen, Y.K. Zhang, Anal. Bioanal. Chem. 405 (2013) 6639–6648.
[26] K. Du, J. Chromatogr A 1374 (2014) 164–170.
[27] Y. Xiao, J. Guo, D. Ran, Q. Duan, J. Crommen, Z. Jiang, J. Chromatogr. A 1400 (2015) 47–53.
[28] P. Gagnon, F. Hensel, S. Lee, S. Zaidi, J. Chromatogr. A 1218 (2011) 2405–2412.
[29] T.V. Gupalova, O.V. Lojkina, V.G. Palagnuk, A.A. Totolian, T.B. Tennikova, J. Chromatogr. A 949 (2002) 185–194.
[30] R. Bandari, J. Kuballa, M.R. Buchmeiser, J. Sep. Sci. 36 (2013) 1169–1175.
[31] P. Levkin, P. Eeltnik, T.R. Stratton, R. Brennen, K. Robotti, H. Yin, K. Killeen, F. Svec, J.M.J. Fréchet, J. Chromatogr. A 1200 (2008) 55–61.
[32] C. Aquah, C.K.S. Moy, M.K. Danquah, C.M. Ongkudon, J. Chromatogr. B 1015-1016 (2016) 121–134.
[33] E. Vlakh, A. Novikov, G. Vlasov, T. Tennikova, J. Pept. Sci. 10 (2004) 719–730.
[34] I.V. Kalashnikova, N.D. Ivanova, T.B. Tennikova, Anal. Chem. 80 (2008) 2188–2198.
[35] S.L. Williams, M.E. Eccleston, N.K.H. Slater, Biotechnol. Bioeng. 89 (2005) 783.
[36] V.A. Korzhikov, E.G. Vlakh, C. Kasper, T.B. Tennikova, Russ. J. Bioorg. Chem. 37 (2011) 270–276.
[37] S. Roeker, S. Boehm, S. Diederichs, F. Bode, A. Quade, V. Korzhikov, M. van Griensven, T.B. Tennikova, C. Kasper, J. Biomed. Mater. Res. B, Appl. Biomater. 91 (2009) 153–162.
[38] J. Krenkova, N.A. Lacher, F. Svec, J. Chromatogr. A 1216 (2009) 3252–3259.
[39] J. Krenkova, N.A. Lacher, F. Svec, Anal. Chem. 81 (2009) 2004–2012.
[40] S. Wu, L. Sun, J. Ma, K. Yang, Z. Liang, L. Zhang, Y. Zhang, Talanta 83 (2011) 1748–1753.
[41] P.S. Sharma, Z. Iskierko, A. Pietrzyk-Le, F. D'Souza, W Kutner, Electrochem. Commun. 50 (2015) 81–87.
[42] E.A. Kataev, C. Muller, Tetrahedron 70 (2014) 137–167.
[43] M.J. Whitkombe, N. Kirsch, I.A. Nichols, J. Mol. Recognit. 27 (2014) 297–401.
[44] E.G. Vlakh, V.A. Korzhikov, T.B. Tennikova, Russ. Chem. Rev. 84 (2015) 952–980.
[45] R. Song, X. Hu, P. Guan, J. Li, N. Zhao, Q. Wang, Mater. Sci. Eng. C 44 (2014) 69–75.
[46] E.G. Vlakh, V.A. Korzhikov, A.V. Hubina, T.B. Tennikova, Russ. Chem. Rev. 84 (2015) 952–980.
[47] J. Janata, Principles of Chemical Sensors, second ed. Springer, New York, 2009; L. Uzun, A.P.F. Turner, Biosens. Bioelectron. 76 (2006) 131–144.
[48] Z. Iskierko, P.S. Sharma, K. Bartold, A. Pietrzyk-Le, K. Noworyta, W. Kurtner, Biotechnol. Adv. 34 (2016) 30–46.
[49] P.S. Sharma, Z. Iskierko, A. Pietrzyk-Le, F. D'Souza, W. Kutner, Electrochem. Commun. 50 (2015) 81–87.
[50] W.J. Cheong, S.H. Yang, F. Ali, J. Sep. Sci. 36 (2013) 609–628.
[51] L. Mergola, S. Scorrano, R. Del Sole, M.R. Lazzoi, G. Vasapollo, Biosens. Bioelectron. 40 (2013) 336–341.
[52] A.M. Attaran, N. Mohammadi, M. Javanbakht, B. Akbari-Adergani, J. Chromatogr. Sci. 52 (2014) 730–738.
[53] M.M. Moein, M. Javanbakht, B. Akbari-adergani, Talanta 121 (2014) 30–36.
[54] T. Panasyuk-Delaney, V.M. Mirsky, V.S. Otto, Electroanalysis 14 (2002) 221–224.
[55] P.S. Sharma, A. Wojnarowicz, M. Sosnowska, T. Benincori, K. Noworyta, F. D'Souza, W. Kutner, Biosens. Bioelectron. 77 (2016) 565–572.
[56] M. Dabrowski, P.S. Sharma, Z. Iskierko, K. Noworyta, M. Cieplak, W. Lisowski, S. Oborska, A. Kuhn, W. Kutner, Biosens. Bioelectron. 79 (2016) 627–635.
[57] Y. Li, L. Tan, H. Li, Z. Hu, X. Zuo, Y. Tang, J. Chromatogr. A 1324 (2014) 190–197.
[58] Q. Zhang, L. Jing, J. Zhang, Y. Ren, Y. Wang, Y. Wang, T. Wei, B. Liedberg, Anal. Biochem. 463 (2014) 7–14.
[59] L. Meng, P. Meng, Q. Zhang, Y. Wang, Anal. Chim. Acta 771 (2013) 86–94.
[60] L. Meng, P. Meng, B. Tang, Q. Zhang, Y. Wang, Forensic Sci. Int. 231 (2013) 6–12.
[61] P.D. Howes, R. Chandrawati, M.M. Stevens, Science 346, 2014, http://dx.doi.org/10.1126/science.1247390.
[62] E. Remhult, F. Höök, Sensors 15 (2015) 1635–1675.
[63] J. Kirsch, C. Siltanen, Q. Zhou, A. Revzin, A. Simonian, Chem. Soc. Rev. 42 (2013) 8733–8368.
[64] M. Perfézou, A. Turnerb, A. Merkoçi, Chem. Soc. Rev. 41 (2012) 2606–2622.
[65] C. Costa, M. Abal, R. López-López, L. Muinelo-Romay, Sensors 14 (2014) 4856–4875.
[66] M. Zhao, X.D. Chai, J. Han, G.F. Gui, R. Yuan, Y. Zhuo, Anal. Chim. Acta 846 (2014) 36–43.
[67] R. Malhotra, V. Patel, B.V. Chikkaveeraiah, B.S. Munge, S.C. Cheong, R.B. Zain, M.T. Abraham, D.K. Dey, J.S. Gutkind, J.F. Rusling, Anal. Chem. 84 (2012) 6249–6955.
[68] V. Perumal, U. Hashim, J. Appl. Biomed. 12 (2014) 1–15.
[69] S. de Picciotto, B. Imperiali, L.G. Griffith, K.D. Wittrup, Anal. Biochem. 460 (2014) 9–15.
[70] L. Choulier, V.V. Shvadchak, A. Naidoo, A.S. Klymchenko, Y. Mely, D. Altschuh, Anal. Biochem. 401 (2010) 188–195.
[71] E. Burcu Bahadır, M.K. Sezgintürk, Talanta 132 (2015) 162–174.
[72] F. Long, A. Zhu, H. Shi, Sensors 13 (2013) 13928–13948.
[73] G. Garcia-Martinez, E.A. Bustabad, H. Perrot, C. Gabrielli, B. Bucur, M. Lazerges, D. Rose, L. Rodriguez-Pardo, J. Farina, C. Compère, A. Arnau Vives, Sensors 11 (2011) 7656–7664.
[74] K. Ramanathan, B. Danielsson, Biosens. Bioelectron. 16 (2001) 417–423.
[75] C. Kaittanis, T. Banerjee, S. Santra, O.J. Santiesteban, K. Teter, J.M. Perez, Bioconjug. Chem. 22 (2011) 307–314.
[76] K. Rogers, Mol. Biotechnol. 14 (2000) 109–129.
[77] M.A. Arugula, A. Simonian, Meas. Sci. Technol. 25 (2014) 032001.
[78] R. Singh, M. Das Mukherjee, G. Sumana, R.K. Gupta, S. Sood, B.D. Malhotra, Sensors Actuators B 197 (2014) 385–404.
[79] L. Wanbo, X. Jiang, J. Xue, Z. Zhou, J. Zhou, Biosens. Bioelectron. 68 (2015) 468–474.
[80] J. Treviño, A. Calle, J.M. Rodríguez-Frade, M. Mellado, L.M. Lechuga, Clin. Chim. Acta 403 (2009) 56–62.
[81] A.G. Jay, A.N. Chen, M.A. Paz, J.P. Hung, J.A. Hamilton, J. Biol. Chem. 290 (2015) 4590–4603.
[82] K. Pimková, M. Bocková, K. Hegnerová, J. Suttnar, J. Čermák, J. Homola, J.E. Dyr, Anal. Bioanal. Chem. 402 (2012) 381–387.
[83] N.E. Azmi, N.I. Ramli, J. Abdullah, M. Azmi, A. Hamid, H. Sidek, S. Abd Rahman, N. Ariffin, N.A. Yusof, Biosens. Bioelectron. 67 (2015) 129–133.
[84] Y. Xu, B. Jiang, J. Xie, Y. Xiang, R. Yuan, Y. Chai, Talanta 128 (2014) 237–2341.
[85] J. Shi, J. Guo, G. Bai, C. Chan, X. Liu, W. Ye, J. Hao, S. Chen, M. Yang, Biosens. Bioelectron. 65 (2015) 238–244.
[86] N.P. Sardesai, J.C. Barron, J.F. Rusling, Anal. Chem. 83 (2011) 6698–6703.
[87] Y. He, Y. Chai, R. Yuan, H. Wang, L. Bai, N. Liao, Analyst 139 (2014) 5209–5214.
[88] X.N. Xu, Z. Wen, W.J. Brownlow, J. Electroanal. Chem. 688 (2013) 53–60.
[89] Y. Zhou, Y. Zhuo, N. Liao, Y. Chai, R. Yuan, Talanta 129 (2014) 219–226.
[90] T.T. Zhang, H.M. Zhao, X.F. Fan, S. Chen, X. Quan, Talanta 131 (2015) 379–385.
[91] X. Jiang, H. Wang, R. Yuan, Y. Chai, Biosens. Bioelectron. 63 (2015) 33–38.
[92] L. Pasquardini, L. Pancheri, C. Potrich, A. Ferri, C. Piemonte, L. Lunelli, L. Napione, V. Comunanza, M. Alvaro, L. Vanzetti, F. Bussolino, C. Pederzolli, Biosens. Bioelectron. 68 (2015) 500–507.
[93] C. Shi, Q. Meng, D.W. Woo, J. Mol. Endocrinol. 52 (2014) 55–66.
[94] S.F. Chou, W.L. Hsu, J.M. Hwang, C.Y. Chen, Anal. Chim. Acta 453 (2002) 181–189.
[95] S. Zhang, H. Bai, J. Luo, P. Yang, J. Cai, Analyst 139 (2014) 6259–6265.
[96] M. Mujika, A. Zuzuarregui, S. Sánchez-Gómez, G. Martínez de Tejada, S. Arana, E. Pérez-Lorenzo, J. Biotechnol. 186 (2014) 162–168.
[97] M.Ç. Canbaz, C.S. Simşek, M.K. Sezgintürk, Anal. Chim. Acta 814 (2014) 31–38.
[98] R. Li, H. Huang, L. Huang, Z. Lin, L. Guo, B. Qiu, G. Chen, Electrochim. Acta 109 (2013) 233–237.
[99] Y.T. Tung, M.F. Wu, G.J. Wang, S.L. Hsieh, Nanomed. Nanotechnol. 10 (2014) 1335–1341.
[100] R. Ohno, H. Ohnuki, H. Wang, T. Yokoyama, H. Endo, D. Tsuya, M. Izumi, Biosens. Bioelectron. 40 (2013) 422–426.
[101] R. Montes, M. Baeza, F. Céspedes, Microchem. J. 119 (2015) 66–74.
[102] S. Mariani, M. Minunni, Anal. Bioanal. Chem. 406 (2014) 2303–2323.
[103] R.J. Förster, P. Bertoncello, T.E. Keyes, Ann. Rev. Anal. Chem. 2 (2009) 359–385.
[104] A. Hasan, M. Nurunnabi, M. Morshed, A. Paul, A. Polini, T. Kuila, M. Al Hariri, Y. Lee, A.A. Jaffa, Biomed. Res. Int. (2014), http://dx.doi.org/10.1155/2014/307519.
[105] S. Cortès, C.L. Villiers, P. Colpo, R. Couderc, C. Brakha, F. Rossi, P.N. Marche, M.B. Villiers, Biosens. Bioelectron. 26 (2011) 4162–4168.
[106] R.J. Schutte, A. Parisi-Amon, W.M. Reichert, J. Biomed. Res. A 88 (2009) 128–139.
[107] G.B. Schneider, A. English, M. Abraham, R. Zaharias, C. Stanford, J. Keller, Biomaterials 25 (2004) 3023–3028.
[108] L. Bacakova, E. Filova, M. Parizek, T. Ruml, V. Svorcik, Biotech. Adv. 29 (2011) 739–767.
[109] A.E. Rodda, L. Meagher, D.R. Nisbet, J.S. Forsythe, Prog. Polym. Sci. 39 (2014) 1312–1347.
[110] N.D. Evans, E. Gentleman, J. Mater. Chem. B 2 (2014) 2345–2356.
[111] B. Finke, F. Hempel, H. Testrich, A. Artemenko, H. Rebl, O. Kylián, J. Meihsner, H. Biederman, B. Nebe, K.-D. Weltmann, K. Schröder, Surf. Coat. Technol. 205 (2011) 520–524.
[112] A.R. Boccaccini, M. Erol, W.J. Stark, D. Mohn, Z. Hong, J.F. Mano, Compos. Sci. Technol. 70 (2010) 1764–1766.
[113] F. He, W. Ren, X. Tian, W. Liu, S. Wu, X. Chen, Mater. Sci. Eng. C 64 (2016) 117–123.
[114] F. Khan, M. Tanaka, S.R. Ahmad, J. Mater. Chem. B 3 (2015) 8224–8249.
[115] H. Cui, Y. Liu, M. Deng, X. Pang, P. Zhang, X. Wang, X. Chen, Y. Wei, Biomacromolecules 13 (2012) 2881–2889.
[116] R.L. Di Marco, S.C. Heilshorn, Adv. Mater. 24 (2012) 3923–3940.
[117] E.G. Vlakh, E.F. Panarin, T.B. Tennikova, K. Suck, C. Kasper, J. Biomed. Mater. Res. 75 (2005) 333–341.

[118] Y.L. Cui, X. Hou, A.D. Qi, X.H. Wang, H. Wang, K.Y. Cai, Y. Ji Yin, K. De Yao, J. Biomed, Mater. Res. A 66 (2003) 770–778.
[119] V. Korzhikov, S. Roeker, E. Vlakh, C. Kasper, T. Tennikova, Bioconjug. Chem. 19 (2008) 617–625.
[120] G. Wu, P. Li, H. Feng, X. Zhang, P.K. Chu, J. Mater. Chem. B 3 (2015) 2024–2042.
[121] K.G. Battiston, J.W.C. Cheung, D. Jain, J.P. Santerre, Biomaterials 35 (2014) 4465–4476.
[122] C.E. Clarkin, R.J. Emery, A.A. Pitsillides, C.P.D. Wheeler-Jones, J. Cell. Physiol. 214 (2008) 537–544.
[123] T. Korff, S. Kimmina, G. Martiny-Baron, H. Augustin, FASEB J. 15 (2001) 447–457.
[124] D. Steiner, F. Lampert, G.B. Stark, G. Finkenzeller, J. Orthop. Res. 30 (2012) 1682–1689.
[125] J.E. McBane, K. Cai, R.S. Labow, J.P. Santerre, Acta Biomater. 8 (2012) 488–501.
[126] R.E. Unger, A. Sartoris, K. Peters, A. Motta, C. Migliaresi, M. Kunkel, U. Bulnheim, J. Rychly, C.J. Kirkpatrick, Biomaterials 28 (2007) 3965–3976.
[127] T. Xu, W. Zhao, J.M. Zhu, M.Z. Albanna, J.J. Yoo, A. Atala, Biomaterials 34 (2013) 130–139.
[128] A. Shekaran, A.J. Garcia, Biochim. Biophys. Acta 1810 (2011) 350–360.
[129] B. Renga, D. Francisci, E. Schiaroli, A. Carino, S. Cipriani, C. D'Amore, A. Sidoni, R. Del Sordo, I. Ferri, M. Lucatelli, B. Lunghi, F. Baldelli, S. Fiorucci, PLoS One 9 (2014), e94798, http://dx.doi.org/10.1371/journal.pone.0094798.
[130] J.A. Hubbell, S.P. Massia, N.P. Desai, P.D. Drumheller, Biotechnology 6 (1991) 568–572.
[131] M. Yamada, Y. Kadoya, S. Kasai, K. Kato, M. Mochizuki, N. Nishi, N. Watanabe, H.K. Kleinman, Y. Yamada, M. Nomizu, FEBS Lett. 530 (2002) 48–52.
[132] T. Maeda, K. Titani, K. Sekiquchi, J. Biochem. 115 (1994) 182–189.
[133] L. Aucoin, C.M. Griffith, G. Pleizier, Y. Deslandes, H. Sheardown, J. Biomater. Sci. Polym. Ed. 13 (2002) 447–462.
[134] M. Barczyk, S. Carracedo, D. Gullberg, Cell Tissue Res. 339 (2010) 269–280.
[135] S. Fiorucci, A. Mencarelli, B. Palazzetti, A.G. Sprague, E. Distrutti, A. Morelli, T.I. Novobrantseva, G. Cirino, V.E. Koteliansky, A.R. de Fougerolles, Immunity 17 (2002) 769–780.
[136] F. Causa, E. Battista, R. Della Moglie, D. Guarnieri, M. Iannone, P.A. Netti, Langmuir 26 (2010) 9875–9884.
[137] B. Liu, Y. Liu, J.J. Riesberg, W. Shen, J. Am. Chem. Soc. 132 (2010) 13630–13632.
[138] K. Konstantopoulos, S. Kukreti, L.V. McIntire, Adv. Drug Deliv. Rev. 33 (1998) 141–164.
[139] D. Wang, W.M. Toyofuku, A.M. Chen, M.D. Scott, Biomaterials 32 (2011) 9494–9503.
[140] N.A. Rossi, I. Constantinescu, R.K. Kainthan, D.E. Brooks, M.D. Scott, J.N. Kizhakkedathu, Biomaterials 31 (2010) 4167–4178.
[141] H. Jung, A.D. Robison, P.S. Cremer, J. Struct. Biol. 168 (2009) 90–94.
[142] M. Arnold, V. Hirschfeld-Warneken, T. Lohmüller, P. Heil, J. Blümmel, E.A. Cavalcanti-Adam, M. López-García, P. Walther, H. Kessler, B. Geiger, J.P. Spatz, Nano Lett. 8 (2008) 2063–2069.
[143] X. Wang, K. Ye, Z. Li, C. Yan, J. Ding, Organogenesis 9 (2013) 280–286.
[144] H. Yang, W.J. Kao, Int. J. Nanomedicine 2 (2007) 89–99.
[145] G. Maheshwari, G. Brown, D.A. Lauffenburger, A. Wells, L.G. Griffith, J. Cell. Sci. 113 (2000) 1677–1686.
[146] C. Salinas, K. Anseth, Biomaterials 29 (2008) 2370–2377.
[147] M. Tsurkan, K. Chwalek, K. Levental, U. Freudenberg, C Werner, Macromol. Rapid Commun. 31 (2010) 1529–1533.
[148] R. Nakaoka, Y. Hirano, D.J. Mooney, T. Tsuchiva, A. Matsuoka, J. Artif. Organs 3 (2013) 284–293.
[149] T. Shahal, B. Geiger, I.E. Dunlop, J.P. Spatz, Biointerphases 7 (2012) 23, http://dx.doi.org/10.1007/s13758-012-0023-0.
[150] K.A. Kilian, M. Mrksich, Angew. Chem. Int. Ed. 51 (2012) 4891–4895.
[151] L. Li, J.R. Klim, R. Derda, A.H. Courtney, L.L. Kiessling, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 11745–11750.
[152] Y.Z. Zhang, D.B. Moheban, B.R. Conway, A. Bhattacharayya, R.A. Segal, J. Neurosci. 20 (2000) 5671–5678.
[153] V.H. Fan, K. Tamama, A. Au, R. Littrell, L.B. Richardson, J.W. Wright, A. Wells, L.G. Griffith, Stem Cells 25 (2007) 1241–1251.
[154] B.K. Mann, R.H. Schmedlen, J.L. West, Biomaterials 22 (2001) 439–444.
[155] M.K. Horne, D.R. Nisbet, J.S. Forsythe, C.L. Parish, Stem Cells Dev. 19 (2010) 843–852.
[156] P.C. Bessa, R. Machado, S. Nürnberger, D. Dopler, A. Banerjee, A.M. Cunha, J.C. Rodríguez-Cabello, H. Redl, M. van Griensven, R.L. Reis, M. Casal, J. Control. Release 142 (2010) 312–318.
[157] Y.Z. Zhang, D.B. Moheban, B.R. Conway, A. Bhattacharayya, R.A. Segal, J. Neurosci. 20 (2000) 5671–5678.
[158] B.L. MacInnis, R.B. Campenot, Science 295 (2002) 1536–1539.
[159] K. Moore, M. McSween, M. Shoichet, Tissue Eng. 12 (2006) 267–278.
[160] H. Toda, M. Yamamoto, H. Kohara, Y. Tabata, Biomaterials 32 (2011) 6920–6928.
[161] B.L. Beckstead, J.C. Tung, K.J. Liang, Z. Tavakkol, M.L. Usui, J.E. Olerud, C.M. Giachelli, J. Biomed. Mater. Res. A 91 (2009) 436–446.
[162] B. Varnum-Finney, L. Xu, C. Brashem-Stein, C. Nourigat, D. Flowers, S. Bakkour, W.S. Pear, I.D. Bernstein, Nat. Med. 6 (2000) 1278–1281.
[163] S. Taqvi, L. Dixit, K. Roy, J. Biomed. Mater. Res. A 79 (2006) 689–697.
[164] J. Lee, N.A. Kotov, Small 5 (2009) 1008–1013.
[165] J.J. Moon, S.H. Lee, J.L. West, Biomacromolecules 8 (2007) 42–49.
[166] S. Anderson, B. Shergill, Z. Barry, E. Manousiouthakis, T. Chen, E. Botvinick, M.O. Platt, M.L. Iruela-Arispe, T. Segura, Integr. Biol. 3 (2011) 887–896.
[167] P. Liu, Y. Zhao, Y. Yan, Y. Hu, W. Yang, K. Cai, Mater. Sci. Eng. C 55 (2015) 1–7.
[168] J.L. Drury, D.J. Mooney, Biomaterials 24 (2003) 4337–4351.
[169] K. Vulic, M. Shoichet, J. Am. Chem. Soc. 134 (2012) 882–885.
[170] C.C. Lin, K.S. Anseth, Adv. Funct. Mater. 19 (2009) 2325–2331.
[171] P. Coria, H. Yagi, Y. Kitagawa, Z. Meqeed, Y. Nahmias, R. Sheridan, M.L. Yarmush, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 1034–1039.
[172] E. Hadjipanayi, A.T. Bauer, P. Moog, B. Salgin, H. Kuekrek, B. Fersch, U. Hopfner, T. Messner, A. Schlüter, M. Ninkovic, H.G. Machens, A.F. Schilling, J. Control Release 169 (2013) 91–102.
[173] A. Simmons, E. Alsberg, S. Hsiong, W.J. Kim, D.J. Mooney, Bone 35 (2004) 562–569.
[174] H. Kohara, Y. Tabata, Biomaterials 32 (2011) 5726–5732.
[175] N.J. Shah, M.L. Macdonald, Y.M. Beben, R.F. Padera, R.E. Samuel, P.T. Hammond, Biomaterials 32 (2011) 6183–6193.
[176] J.M. Kanczler, P.J. Ginty, L. White, N.M. Clarke, S.M. Howdle, K.M. Shakesheff, R.O.C. Oreffo, Biomaterials 31 (2010) 1242–1250.

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