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Aptamer-Mediated Selective Protein Affinity to Improve Scaffold Biocompatibility

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Additional information is available at the end of the chapter

Abstract

Protein adsorption on surfaces occurs shortly after scaffold insertion. This process is of pivotal importance to achieve therapeutic success in tissue engineering (TE), and favorable proteins should be adsorbed at the interface without unfolding to preserve their structure and function. Protein misfolding at the interface is a common phenomenon, which can impair cell adhesion and scaffold colonization. Many efforts have been done to improve scaffold biocompatibility by ameliorating protein adsorption, but with poor results. In the present chapter, we propose the use of a novel class of molecules, aptamers, to improve scaffold biocompatibility. Aptamers are small, single stranded oligonucleotides, which specifically bind to a target molecule: they work as antibodies, but without many of the drawbacks associated to the use of antibodies. We propose to immobilize aptamers on scaffolds to retain specific proteins, acting as docking points to guide cell activity. In particular, we show the results obtained by enriching different polymeric scaffolds with aptamers against human fibronectin, a naturally abundant protein in tissues, which plays a pivotal role in cell adhesion. We demonstrate that scaffold enrichment with aptamers lead to a better colonization of the substrate from cells. The results we obtained pave the way to the possibility of further investigating the role of aptamers as useful molecules to improve scaffold biocompatibility in the contest of tissue engineering.

Keywords: aptamers, biocompatibility, fibronectin, scaffold, SELEX, tissue engineering

1. Introduction

Regenerative medicine (RM) is a therapeutic approach that aims to restore structure and function of damaged tissues and organs, in particular to find a solution for those that become permanently damaged and untreatable [1].

RM can be potentially applied to different tissues [2], and one of the most promising fields is that related to bone [3, 4].

Tissue regeneration is a complex task that encompasses completely restoring the lost structure, including its micro-architecture and consequently its functionality. As for bone regeneration, optimal healing is achieved when certain prerequisites are met, namely, osteoinduction, osteoconduction, osteogenesis, and mechanical stability [5].

Osteoinduction is the process that allows the recruitment and stimulation of immature pre-osteoblastic cells to mature osteoblasts and to produce new bone [6]. This phenomenon is regulated by a class of molecules known as inductive agents, mainly represented by bone morphogenetic proteins (BMPs) [3]. As a consequence of osteoinduction, osteogenesis can be achieved. Osteogenesis is carried out by osteoblasts, and consists in the formation of new bone. To improve the outcome of bone regeneration, biomaterials are often used to fill the gaps created by lost tissue. Such biomaterials must be osteoconductive, i.e., capable of supporting bone deposition on their surface [6]. Finally, mechanical stability of the healing site is the fourth factor to consider in order to reach regeneration of sound bone and avoid formation of fibrous tissue [5].

RM for bone tissue currently includes four approaches: molecular, cellular, use of bone substitutes, and tissue engineering (TE).

Progresses in molecular biology and a deeper knowledge of the mechanisms of fracture healing at a molecular level have allowed for the identification of a large number of key molecules that can be used locally or systematically to enhance bone repair [7]. Autologous cells can be an alternative or complementary choice for healing bone fracture. Mesenchymal stem cells (MSCs) have been proposed as a useful in regenerative interventions. MSCs can be collected from bone marrow [8], from peripheral blood [9], or from adipose tissue [10, 11]. Further possibilities to harvest MSCs in dental applications could be other types of stem cells directly isolated from oral tissues such as the dental pulp (DPSCs) or the periodontal ligament (PDLSCs) [12–14]. As mentioned before, biomaterials have also been proposed as a tool to provide a substrate for new bone cells to deposit new bone, acting as gap fillers and osteoconductive scaffold. A wide number of synthetic bone substitutes are now available including hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), and calcium-phosphate cements, glass ceramics, and biocompatible metals [15, 16].

These different approaches are often combined and the investigation of the optimal conditions and tools to regenerate a tissue created a field called tissue engineering.

1.1. Tissue engineering

Tissue engineering (TE) was first defined in 1988 at the first *TE symposium* in California, as "*an interdisciplinary field of research that applies the principles of engineering and the life sciences towards the development of biological substitutes that restore, maintain and improve tissue function*". It has been demonstrated that TE offers great potential in clinical applications [17, 18], and, in particular, bone tissue engineering seems to harbor a great potential. At present, bioabsorbable scaffolds combined with bone-marrow aspirate and osteoinductive factors (BMPs) have yielded promising results [16], and, more recently, the applicability of a β-TCP scaffold seeded with autogenous bone-marrow cells for bone reconstruction has been shown in a sheep model [19]. Moreover, TE has been used to improve fracture healing and to augment the bone-prosthesis interface in arthroplasty, with promising results and safety [20, 21].

1.1.1. Scaffold

Scaffolds are a central concept in TE. They are 3D porous structures designed to promote cell adhesion, proliferation, and extracellular matrix deposition in order to allow for the restoration of damaged tissue [22].

Scaffolds can be divided into biological and synthetic materials. Biological scaffolds are derived from human and animal tissues, whereas synthetic ones are made of artificial biomaterials [23]. As materials of biological origin, although often possessing favorable characteristics, suffer from scarce availability, safety concerns and sometimes possibility of inflammatory or even immune responses, synthetic biomaterials have been the center of increasing attention. The state of art on scaffolds has evolved over the last years and involves the employment of natural or synthetic polymers. Collagen is the most abundant polymer in tissues and, as a consequence, among the most investigated material for the production of natural-derived scaffolds [24–26]. Together with collagen, chitosan, alginate, and cellulose are promising biomaterial for bone tissue engineering applications [27–30]. Among the synthetic polymers used for scaffold fabrication, polylactic-co-glycolic acid (PLGA) and polycaprolactone (PCL) are probably the most studied [31]. However, their characteristics for TE applications are still suboptimal compared with those of natural polymers [4]. Alternatively to the use of polymers, calcium phosphate, apatite forms, and bioglasses find wide application in bone engineering [32]. Regardless of their chemistry, the main feature scaffolds should possess is biocompatibility.

2. Biocompatibility

The concept of biocompatibility is widely used within biomaterial science, but it is still uncertain what it really means. When it was first used in the early 1940s, a material was considered biocompatible if it could be placed in contact with tissues without altering them: a biocompatible material was conceived to be ideally inert. However, as research progressively revealed that a true biological inertia is not possible, because any thing that enters in contact with a tissue induces a non-self response from the host immune system, the concept of

biocompatibility had to be necessarily reviewed. For years materials were considered biocompatible if they were non-toxic, non-immunogenic, non-carcinogenic, non-irritant, and so on against human body. During the 1980s, new evidences brought about another change of view and lead to a more modern definition of biocompatibility. First, it was clear that materials always react with tissues and that they are not inert. Second, it was shown that biological responses to biomaterial are different across tissues, and that the tissue itself affects material biocompatibility. Third, the scientific community realized that some clinical situation require that materials get degraded and removed from the host after accomplishing their function [33]. Accordingly to these concepts, a widely accepted definition of biocompatibility was outlined at the Consensus Conference in Boston in 1987 as follows: "Biocompatibility refers to the ability of a material to perform with an appropriate host response in a specific situation" [34].

In conclusion, focusing on this definition, a material is inserted into a tissue to perform a function, not simply lie inertly, and tissue responses to the material have to be adequate to the specific desired applications [35].

Biocompatibility as defined above is a pivotal concept for TE and scaffolds fabrication. A scaffold can be considered for *in vivo* application if it has been proven to be biocompatible *in vitro*, i.e., if it can support cell adhesion and proliferation. Cellular responses, in turn, heavily depend on protein adsorption on the scaffold surface. Protein adsorption on materials is a spontaneous phenomenon that can be accompanied by protein denaturation, i.e., alteration of protein conformation and function [36]. Protein denaturation on to surfaces may occur for different reasons, mainly due to the chemical and physical characteristics of the material, and for that, a series of methods to enhance the biocompatibility of the surfaces have been developed.

2.1. Modern approaches to enhance scaffold biocompatibility

It has been solidly established that shortly after implantation biomaterials are covered with a thin layer of host proteins, and it is believed that the state of adsorbed proteins play a key role in scaffold colonization from cells [37]. Therefore, controlling the amount, composition and conformation of adsorbed proteins is a viable approach to obtain a highly biocompatible surface [38]. In recent years, several strategies have been developed to guide protein adsorption and thus to improve cell adhesion, including immobilizing short fragment or proteins on scaffolds, or chemically and physically modifying scaffold surfaces.

2.1.1. Chemical and physical treatments

It has been demonstrated that some proteins bind preferentially certain chemical groups. For example it has been shown that fibrinogen binds methyl (–CH3) functionalized surfaces, but not carboxy (–COOH) ones, whereas the hydroxy (–OH) groups enhance the affinity for albumin over fibrinogen [39–41]. Therefore, the first strategy developed to control protein adsorption on scaffolds was enriching surfaces with functional groups, by combining chemical and physical treatments.

Chemical graft modification entails surface activation through different methods, such as chemical reactions or UV, plasma, and ozone exposure [42], followed by covalent grafting of the desired functional groups. Chemical grafting has been used to improve hemocompatibility of vascular grafts by enriching them with heparin and polyethylene glycol (PEG or PEO). The drawbacks of this approach include the loss of protein mobility at the material surface, because they are covalently bound and the possible release of toxic monomers [38].

To overcome issues associated to chemical graft deposition, self-assembled monolayers (SAMs) were developed. SAMs was widely used to study *in vivo* responses of implanted biomaterials in the past, although nowadays is limited to gold- and silver-coated surfaces [38, 43, 44].

An increasingly popular method to graft surfaces with functional groups is plasma modification. Plasma is considered the fourth state of matter and it is obtained when gases are excited by specific electromagnetic frequencies. Plasma modification is cheap and seems to be very effective, but it is still being currently investigated for the development of biomedical devices, including metals, polymers, and ceramics [38, 45].

2.1.2. Immobilization of RGD and other recognition sequences for integrins

One of the most recent approaches developed to enhance scaffold biocompatibility is the surface immobilization of small peptides able to mimic proteins involved in cell adhesion, to enrich scaffolds with docking points for cells (Ruoslahti, 1996). The best investigated peptide is the arginine-glycine-aspartic acid (RGD) motif, an ubiquitous adhesive sequence found in many ECM proteins responsible for their interaction with cellular integrin receptors [46]. Several groups have studied the *in vitro* ability of RGD and related motifs to improve osteoblast adhesion, migration, and gene expression [47–49]. Moreover, coating titanium implants with the RGD peptide has been shown to induce a direct activation of macrophages, osteoblasts, and osteoclasts in rat tibia and femur and in dog femur [50–52].

However, Hennessy et al. enriched hyaluronic acid disks with RGD and observed poor cell adhesion and inhibitory effects of the RGD binding domain, probably due to the fast adsorption of fibronectin, vitronectin and fibrinogen within 30 min, which competed with RGD motifs to bind integrins [53].

2.1.3. Surface coatings

The application of coatings that mimic the ECM could be an alternative method to improving scaffold biocompatibility. In particular, coatings for bone biomaterials should promote the creation of a suitable environment for osteoblast, osteoclasts, and progenitor cells, that promote implant integration, by improving bone/implant contact (BIC) [46]. Coating titanium implants with collagen, which is the most abundant protein in bone tissue, supports *in vitro* adhesion, migration, and differentiation of osteoblasts [54, 55]. Similarly, coatings of hydroxyapatite-based scaffold with chondroitin sulfate (CS), wide spread in cancellous and cortical bone, Hyaluronic acid (HA) or heparin have demonstrated to increase BMPs secretion and consequently osteoblasts differentiation [56, 57].

All the issues connected to the strategy described, prompted us to develop a new method to enhance scaffold biocompatibility by using a novel class of molecules, called aptamers, to improve protein adsorption and cell adhesion.

3. Aptamers

In the 1980s molecular virology revealed that small structured oligonucleotides could bind proteins with high affinity and specificity. That evidence supported the use of oligonucleotides as specific receptors, which 10 years later lead to the discovery of aptamers [58]. The word "aptamer" was first used in 1990 by Ellington and Szostak to describe small RNAs molecules able to bind small organic dyes. It derives from the fusion of the Latin expression "aptus", which means "to fit", and the Greek word "meros", which means "part" [59]. Since then, aptamers have been defined as short oligonucleotides that by adopting specific 3D conformations are able to bind specific and selected targets [60].

Aptamers are mostly short single-stranded or double-stranded DNA or RNA oligonucleotides, usually 20–80 bp long and 6–30 kDa heavy. Aptamers are constituted of a random sequence region at center, flanked by constant designed primer binding sites and the 3′ and 5′ ends. The sequence region in the center is necessary for target recognition (**Figure 1**), which occurs after aptamer 3D adaptation. In this phenomenon intermolecular interactions, such as Van der Waals forces, hydrogen and electrostatic interactions, stabilize the bond between aptamers and their ligands [61, 62]. The aptamers-ligands interactions are highly specific and capable to discriminate among analogues, i.e., enantioselective aptamers have 12.000-fold higher affinity for L-arginine than for D-arginine [63].

Aptamers are thought to be an excellent alternative to the use of monoclonal antibodies (mAB). Compared with antibodies, aptamers overcome their issues and improve their clinical applicability and suitability for industrialization. First of all, aptamers are low-immunogenic and low-toxic molecules, and they are not directly recognized by the human immune system as foreign agents [64–66]. Unlike antibodies, aptamers have a wider target range, they are smaller so that they can easily penetrate into tissue barriers and cells [67]; moreover they can also bind small ligands, such as ions and small molecules, which cannot be recognized by antibodies [68]. Furthermore, aptamers are thermally stable, and can undergo repeated cycles of denaturation/renaturation without damaging their binding efficiency. Finally, aptamer production and eventually modification is cheaper, easier and faster than that of mAB [68].

The interest of research on aptamers is increasing, as shown by the publication rate on this topic, which has exponentially grown in 25 years [61], leading to more than 5500 published articles in the PubMed database including the term "aptamer" in January 2016. In spite of their popularity, their clinical applications are still limited [62], and as of today only one aptamer-based drug has been approved by the US Food and Drug Administration (FDA). Pfizer/Eyetech launched Macugen, a RNA aptamer against VEGF (vascular endothelial growth factor) for the treatment of wet age-related macular degeneration in 2004 [69]. Barriers to the commercialization of aptamers are essentially two. The former is that some *in vitro* generated aptamers do

not elicit a comparable *in vivo* comparable, whereas the latter is that the SELEX process is time-consuming and not very efficient [62]. In spite of these issues, a recent market report projected the global aptamer market to $5.4 billion by 2019 [70].
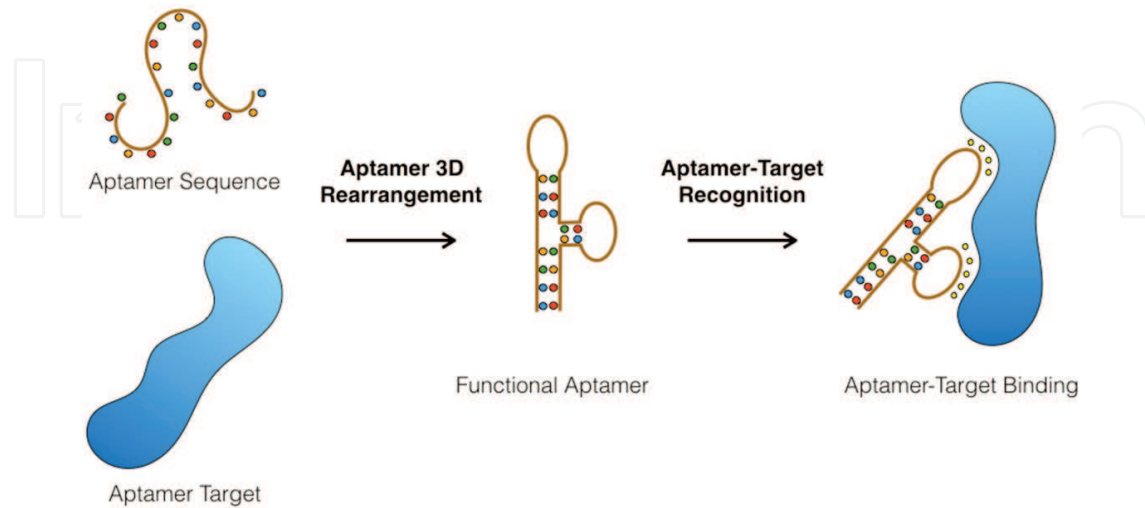

Figure 1. Diagram representing aptamer 3D conformational rearrangement in the presence of the target to form aptamer-target specific complex.

3.1. Aptamers generation

Aptamer selection requires two steps: upstream screening and downstream screening. The upstream screening step identifies full-length aptamers through SELEX (Systematic Evolution of Ligands by EXponential Enrichment), whereas the downstream step aims to isolate the shortest oligonucleotide sequence required for target binding [61].

3.1.1. Upstream screening

In vitro selection or SELEX (Systematic Evolutions of Ligands by EXponential enrichment) is the technique used to isolate aptamers, which was first described by Ellington and Gold in 1990 [59, 71].
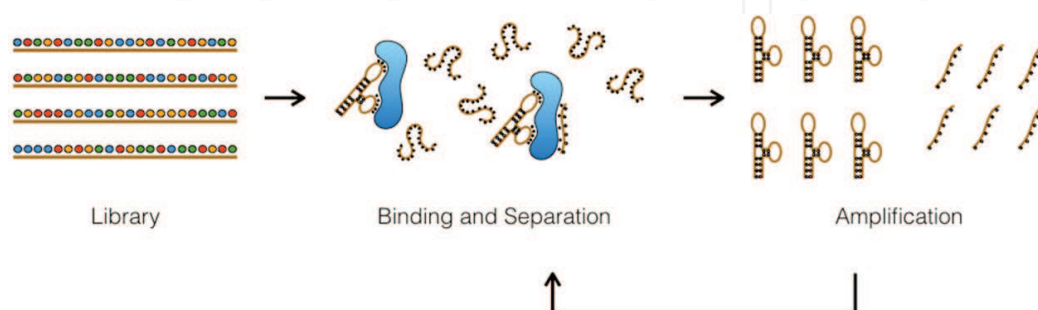

Figure 2. Schematic representation of the SELEX process steps.

The SELEX process consists of three steps, which are then repeated to screen for sequences with higher affinity (**Figure 2**) [58]: (a) the preparation of an initial oligonucleotides pool (library) is followed by (b) the selection of aptamer candidates and by (c) their amplification.

3.1.1.1. Library generation

The whole SELEX process starts with the generation of a synthetic oligonucleotides library, which consists of a pool of ~10^{12} –10^{15} different nucleic acid (ssDNA or RNA) sequences, theoretically able to bind any target molecule. Each sequence represents a possible aptamer candidate and it possesses a central random region, ~25–30 bp long, flanked by two stand-ard primers at the 3′ and 5′ ends [61, 62].

Both ssDNA and RNA libraries can be created and divided in five types, on the basis of the collected sequences. Standard libraries are the most common ones and contain random 20–60 bp long oligonucleotides. Structurally constrained libraries contain oligonucleotides with stable regions, which help aptamers to fold according to a certain secondary structure. Libraries based on a known sequence are constructed by inserting known sequences in the central part of the oligonucleotide. Finally, libraries based on genomic sequences (genomic SELEX) are created by digesting genomic DNA, to find proteins capable to bind it [72].

3.1.1.2. Binding and separation

Once the library is generated, it is incubated with the target. Some of the oligonucleotides in the pool recognize the target and are then considered aptamers (partitioning), whereas unbound sequences are filtered out from the solution (elution) [61].

Several methods are used to discriminate aptamers from other oligonucleotides. The SELEX approach initially employed by Gold and co-workers was based on a nitrocellulose membrane where the T4 DNA polymerase was immobilized [71]. Nowadays, the use of a nitrocellulose membrane is quite out of order because it has some limitations, such as the inability to bind any molecules but proteins and the need to perform at least 12 selection rounds [73, 74]. Alternative strategies have then been developed based on common biochemistry techniques. Chromatographic affinity or magnetic columns are often used for aptamer selection. In the case of chromatographic affinity column, the immobile phase is composed of agarose beads and the targets are immobilized through tags with proteins, such as glutathione *S*-transfer-ase (GST) or His-tag, or through chemical reaction with other molecules. Several aptamers have been selected through this method, however it cannot be applied if the target lacks the tags or the functional group requested for the coupling with the column [75–77]. On the other hand, targets can be immobilized on the surface of magnetic beads and used in magnetic columns, a strategy that is becoming more and more powerful due to the ease of separating aptamers from other nucleotides only by a magnet [78–80]. Furthermore, capillary electro-phoresis has been proposed, because of its speed and high resolution. In fact a successful selection of aptamers can be obtained in a few rounds, i.e., Bowser and co-workers selected aptamer against neuropeptide Y and against human IgE in only four rounds [81, 82]. In addition to the methods described above, aptamers against whole cells have been recently

selected through the Cell-SELEX method. This technique is complex, because cells cannot be immobilized, unlike target molecules; however, several research groups have been successful. Kobatake's group identified the SBC3, a cell lung cancer cell line with a ssDNA aptamers [83]. Previously, Tan's group selected a series of aptamers able to bind two types of ovarian cancer cells [84], whereas Gold et al. isolated an aptamer for the U251 cell line derived from glioblastomas just in 2003 [85].

Further strategies have been implemented to improve SELEX performance, although their efficiency in selecting aptamers is not still clear.

3.1.1.3. Amplification

After the separation of aptamers from a specific nucleotides, they are amplified by PCR, in the case of ssDNA aptamers, or by RT-PCR in the case of RNA aptamers. Consequently, products of amplification are used as a new sub-library for the following selection round [62].

3.1.2. Downstream screening

After the upstream screening or SELEX, selected aptamers are generally ~80 bp long, but the binding region is actually usually 10–15 nucleotides long [68, 86]. As a consequence, redundant and useless nucleotides can be deleted through a process called "aptamer truncation". Many strategies have been tested to minimize aptamer sequences without damaging its binding ability. Most of these strategies are predictive and based on computational biology. Giangrande et al. were able to truncate an RNA aptamer against PSMA (prostate-specific membrane antigen) while preserving its binding activity and functionality, using structure simulations and target docking algorithms. Partial fragmentation was used by Green et al. to select the shortest sequence of DNA aptamers to bind PDGF (platelet-derived growth factor) [87]. Wang and co-workers detected the non-essential region of the hPTK7 (anti-human protein tyrosine kinase 7), hybridized them with complementary oligonucleotides probes [88]; the same approach was used by Duan and co-workers to select the basic region of anti-CD133 aptamer as marker for cancer stem cells [89].

All the methods described for aptamer truncation are effective; however, their application is hindered by their complexity, length, and cost [61].

3.2. Biomedical applications of aptamers

The similarities between aptamers and mABs lead to their applications in various field, including research tools [90], bioassays [91, 92], food safety [93], and environment monitoring [94], as demonstrated by a plethora of reviews recently published on this topic. However, a major field of interest for aptamers is biomedicine, where aptamers can be used as sensors for biomarker discovery, molecular imaging probes, drug delivery systems and drugs, especially in cancer nanomedicine and therapy [58, 61].

3.2.1. Aptamers as potential drugs

Although the most studied aptamers are against thrombin, VEGF and PDGF, aptamer applications range from cancer to infectious pathogens.

3.2.1.1. Therapeutic aptamers in eye disease

The first therapeutic aptamer approved by the FDA was the Pegaptanib, which today is commercially available as Macugen® (Pfizer and Eyetech) [64, 65]. The Pegaptanib is a 27 ribonucleotide pegylated RNA aptamer antagonist of VEGF165 [95]. Since its approval in 2004, the Macugen® has always been used for the treatment of AMD, a degenerative ocular disease that causes vision loss in older adults due to retinal damage. However, the efficacy of this aptamer was then discovered to be important also for the treatment of diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) with promising results in clinical trials [96, 97]. At present, the spectrum of use of Pegaptanib is being broadened to other pathologies such as ischemic diabetic macular edema (MIDME), uveitis, choroidal neovascularization secondary to pathologic myopia, and iris neovascularization [98–100].

The limits of anti-VEGF agents to treat AMD are their inability to promote the regression of new blood vessels, which are the cause for the loss of vision. To bypass this limitation, the E10030 aptamer (Fovista™) was developed by Ophthotech Corp in 2012: the E10030 is a 29 pegylated RNA aptamer able to bind PDGF (platelet-derived growth factor), which regulates pericytes maturation. The combined administration of E10030 with Pegaptanib showed successful neovascular regression in preclinical models [101].

3.2.1.2. Therapeutic aptamers for hemostasis

Thrombin is a wide-studied target for anticoagulation, and its *in vivo* inhibition is a major solution to prevent and treat blood clotting abnormalities [61, 102]. Anti-thrombin aptamer (TBA), a 15 bp oligonucleotide, was first selected in 1992 by Toole et al. and it was the most studied aptamer for clinical applications in 2012 [60, 103]. After the evaluation of TBA efficiency *in vivo* [104], the Nu172 aptamer (ARCA Bipharma) was develop as a potential thrombin inhibitor candidate. Nu172 is a 26 bp aptamer able to prevent fibrinogen cleavage of a-thrombin by interacting with the exosite I. Nu172 is currently in phase II clinical trials to be certified for anticoagulation in invasive medical procedures, coronary artery bypass graft and percutaneous interventions [105].

3.2.1.3. Therapeutic aptamers for cancer

The goal of new therapeutic approaches in Oncology is often to block the neoplastic progression through the inhibition of specific cell-pathways, which lead to cell abnormal proliferation. Several clinical trials have proposed the use of aptamers to specifically bind tumor cells and stop cancer development. The specific cell membrane receptors that can be blocked in tumors are numerous, but only few have been investigated with aptamers. A pivot role is played by nucleolin, a protein which is often over-expressed on the surface of cancer cells and that is firstly involved in cell survival, growth and proliferation, as well as in nuclear trans-

port and transcription [106]. In particular, nucleolin seems to manage the internalization of the tumor-homing F3 peptide and its inhibition affects several signaling pathways responsible for abnormal cell proliferation during cancer progression, such as NF-kB and Bcl-2 pathways [107, 108].

AS1411 (Antisoma, PLC) is a 26 bp long aptamer rich in guanosine and screened for against nucleolin [66, 106]. When AS1411 interacts with surface nucleolin, the complex is internalized and prevents its binding with Bcl-2, thus inducing cell apoptosis. AS1411 has shown good growth-inhibitory properties *in vitro* (**Table 1**) and the ability to be accumulated in tumor tissue [66, 109].

Cell Line	Description	Dose of AS1411 administered	Time of exposure to AS1411
A549	Human epithelial lung carcinoma	1 µmol/l	6 days
DU145	Human epithelial prostate carcinoma	2 µmol/l	6 days
		15 µmol/l	5 days
MDA-MB-231	Human breast adenocarcinoma	15 µmol/l	5 days
MCF-7	Human breast adenocarcinoma	15 µmol/l	5 days
HeLa	Human cervix adenocarcinoma	15 µmol/l	5 days
Primary cells from leukemia	Human leukemia	10 µmol/l	7 days
Primary cells from lymphoma	Human lymphoma	10 µmol/l	7 days

Table 1. Dose administered and time of exposure of different cell lines to AS1411 aptamer, in order to observe growth ihnibition [66, 109].

3.2.1.4. Therapeutic aptamers in microbiology

When aptamers were first described by Ellington and Gold in 1990, their ability to bind viral proteins was clear, and, consequently, their use to treat viral and bacterial diseases has always been investigated [110, 111].

Ebola epidemic of 2014 and other emerging viruses have prompted several research groups to use specific aptamers in the treatment of these diseases by blocking sites essential for virus infectious progression [112–115]. For example, it has been shown that specific aptamers against influenza major targets are able to inhibit or block virus fusion, penetration, and replication [116–120]. Aptamers are also thought to be useful to kill multidrug-resistant (MDR) bacteria *in vivo*, possibly by blocking resistance enzymes such as NMD-1 (New Delhi metallo-β-lactamase) or by inducing the classic pathway of the complement in lieu of antibodies [121–124].

3.2.2. Aptamers as sensors for biomarker discovery

Biomarkers are molecules that change their expression level when physiological conditions are altered, and can thus be used to indicate the progression state of a disease or the risk of

developing it. Biomarkers are therefore a tool with high potentiality for disease screening and early diagnosis. However, a very limited number of biomarkers have been thus far discovered. The use of mABs to identify disease specific targets is often unfeasible, because these targets are frequently cell epitopes and it is impossible to design and select a mAB against an unknown receptor, and aptamer research is moving to fill the gap. Normally, target cells are amplified, collected, and lysed. The lysate is then incubated with aptamers and target proteins go through a comparative proteomic analysis: briefly, they are separated through the SDS-PAGE and analyzed with mass spectrometry [61].

In recent years, many research groups have worked to find aptamers that specifically bind biomarkers. In particular, the tyrosine kinase 7 has been discovered as a potent marker of T-cell acute lymphoblastic leukemia [125], tenascin-C as biomarker for glioblastoma cells [85], the Igμ heavy chain for Burkitt's lymphoma [126], whereas the stress-induced phosphoprotein I for ovarian cancer [127].

3.2.3. Aptamers as molecular imaging probes for diagnostic

Aptamers have also been proposed as detecting agents in diagnostics, both as molecular beacons or as sensors for bio-imaging [58, 61].

In 2001, Hamaguchi et al. developed an aptamer beacon for thrombin. A thrombin aptamer was modified with complementary sequences at 3′ and 5′ ends to form a stem-loop structure. Furthermore, the 5′-end was labeled with a fluorescent moiety, whereas the 3′ with a quencher. In the absence of thrombin, the complementary 3′ and 5′ ends lie in close proximity and this results in fluorescence quenching, whereas in the presence of thrombin, aptamer acquires its 3D specific conformation, moving the fluorophore and the quencher apart, setting off a fluorescence signal in a dose-depends manner [128]. One year later the same approach was proved by Tan and co-workers [129] and then by several research groups [79, 130–132].

The idea of labeling aptamers with fluorophores was pursued also to develop new probes for computerize tomography (CT) and for magnetic resonance imaging (MRI). In addition, this idea seemed appealing in combination with nanomaterials for CT and MRI analysis (i.e., liposomes, quantum dots (QDs), carbon nanotubes, gold and magnetic nanoparticles), to improve *in vivo* imaging and phototermal therapy, thanks to aptamers' accurate targeting and the rapid diffusion through blood circulation of nanomaterials [58]. This approach was used to image C6 cancer cells using a Cy3-labeled aptamer against nucleolin transmembrane protein in 2010 [133]. The same year Min et al. proposed the use of a QDs-aptamer complex specific for PSMA(+) and PSMA(−) (prostate specific membrane antigen) to detect prostate cancer cells. The complex was able to discriminate between prostate cancer cells and normal or other cancer cells [134]. In 2013, Kim et al. immobilized a VEGFR2 (vascular endothelial growth factor receptor 2) aptamer on a magnetic nanocrystal surface for the detection of the angiogenic vasculature in glioblastoma by MRI with high sensitivity and efficiency [135].

3.2.4. Aptamers as drug delivery systems

The ability of aptamers selected through the cell-SELEX to recognize cell antigens have been exploited to deliver a variety of molecules, mainly drugs, into cells [58]. For this purpose, aptamers can be used alone or in combination with other delivery systems, such as polymers or liposomes, in order to enhance their specificity [61].

Building on their previous work on a QDs-aptamer complex specific for PSMA(+) and PSMA(–) (see Section 3.2.3), Min et al. were able to load the construct with doxorubicin, an anticancer drug, and to effectively introduce it inside prostate cancer cells [136]. Levy's group relied on an aptamer against PSMA to introduce a siRNA in prostate cancer cells, which inhibited gene expression within 30 min [137].

To enhance polymers specificity as drug delivery system, they can be funtionalized with aptamers; this strategy has shown to be promising for clinical applications. Farokhzad et al. encapsulated rhodamine-labeled dextran within a nanoparticle of poly (lactic acid)-block-polyethylene glycol copolymer with a terminal carboxylic acid functional group (PLA-PEG-COOH) conjugated to an aptamer against the PSMA antigen of prostate cancer cells. The system was able to enter PSMA over-expressing cells in less than 2 h [138]. The same group further generated a nanoparticle with poly (D,L-lactic-co-glycolic acid)-block-poly (ethylene glycol) (PLGA-b-PEG) copolymer conjugated with the A10 aptamer against PSMA to deliver docetaxel inside cancer cells. This system was tested in vivo, and induced the complete regression of the tumor in five out of seven mice [139]. Following these promising results, several others similar constructs based on the conjugation of polymers and aptamers were efficiently tested [140], and even aptamers conjugated to dendrimers were tested, as reported in a review published in 2011 by Lee et al. [141].

Liposomes were also engineered with aptamers to deliver cisplatin and taxol inside breast cancer cells. The AS1411 aptamer-liposome bioconjugate system efficiently transported cisplatin inside tumorigenic cells, and effectively killed the target cancer cells but not healthy control ones [142]. Moreover, compared with the control liposomes, liposomes conjugated with the AS1411 aptamer and containing taxol, increased the cellular uptake of the construct in the breast cancer cells [143].

Taken together, these results support the use of aptamers as enhancer for drug delivery systems; however, more in vivo evaluation is required to allow their use in clinic [61].

4. Aptamers to enhance scaffold biocompatibility

As mentioned earlier in this chapter (see Section 2.1), one of the most investigated topics in TE is developing new methods to improve scaffold biocompatibility. To reach this goal, scaffolds should be highly dynamic and possess surfaces capable to interact with cells, positively modulating protein adsorption [36].

Several research groups have aimed to reach this goal by immobilizing the RGD peptide binding motif on scaffolds (see Section 2.1.2), by modifying chemically or physically scaffold

surfaces (see Section 2.1.1) or by coating scaffolds with other highly biocompatible materials (see Section 2.1.3).

In this section we propose a new method to improve natural polymeric scaffold biocompatibility, by using ssDNA aptamers screened for against human fibronectin as docking points, to ameliorate the adsorption of fibronectin, a naturally occurring molecule in damaged tissues, which is mainly involved in cell adhesion and in the regeneration process. The correct adsorption of fibronectin may lead to a faster colonization of the scaffold *in vitro* and to an acceleration of the regeneration process *in vivo*. **Figure 3** summarizes the rationale to use aptamers to enrich biomaterials with specific molecules.
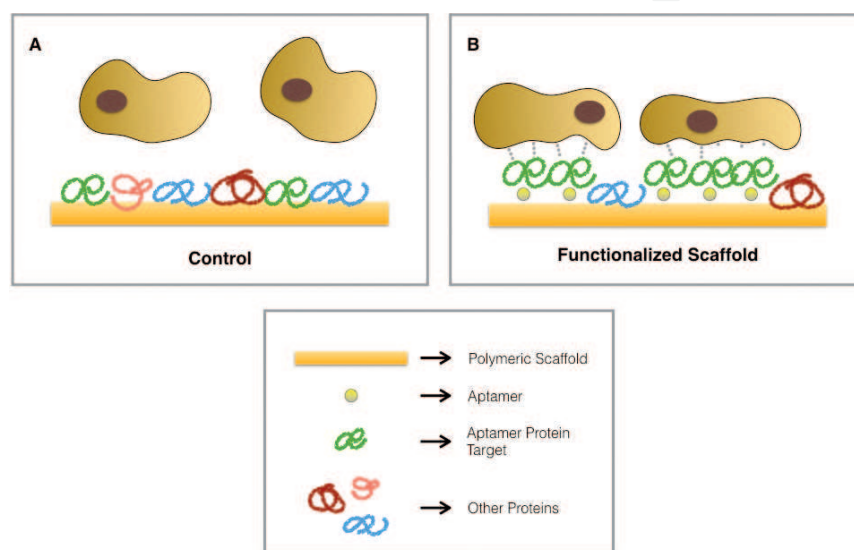

Figure 3. Diagram representing the rationale of functionalizing substrates with aptamers to retain specific proteins. Un-functionalized scaffold adsorbs proteins from the environment based on their availability (A). Aptamer functionalized scaffold specifically binds and retains target protein, by selectively enriching the adsorption for a specific protein (B).

Biomaterial functionalization with aptamers is not new in the literature. Wendel's group pioneered the field in 2007, by coating a vascular prosthesis with aptamers against circulating endothelial progenitor cells (EPCs), to retain specific cells from the bloodstream and quickly create an autologous functional endothelium. Aptamers against EPCs were screened through the Cell-SELEX and covalently grafted on to polydimethylsiloxane (PDMS) and polytetrafluoroethylene (PTFE) substrates. Functionalized scaffolds were incubated with whole porcine blood, washed twice to remove non-specific debris, and stained for CD31 and CD144 by immunofluorescence to identify EPCs. EPCs were observed only on aptamer-grafted prosthesis, whereas no CD31 and CD144 positive cells were retained on control discs [144]. Five years later, Chen et al. designed an artificial ECM using aptamer-grafted polyethylene glycol (PEG) hydrogels: aptamers screened for against cell surface receptors were used as binding sites for cells and they were attached on to the gel through free radical polymerization. It was demonstrated that the amount of cells adhered to hydrogels was proportional to the amount of aptamer incorporated into the hydrogels [145].

Considering those results, we want to show the possibility of enriching natural synthetic scaffolds with aptamers against human Fibronectin to enhance cell adhesion and growth.

For this purpose, we used aptamer screened for against human fibronectin (Base Pair Biotechnologies, Pearland, TX) and modified at their 3′-end with a thiol group and at their 5′-end with biotin.

4.1. Aptamers enhance cell adhesion and proliferation on polymeric scaffolds

Two natural polymer scaffolds were used as substrates: a thiolate hyaluronic acid/polyethylene glycol hydrogel (tHA/PEGDA) and a chitosan modified with D-(+)-raffinose film. tHA/PEGDA gels are 3D matrices normally used for stem cell culture and which offer scant adhesion to cells. For this reason, they are often enriched with adhesion molecules, such as RGD peptides, when firmer adhesion is required. Aptamers were bound to these hydrogels by exploiting the acrylate functional groups of PEGDA, which can easily bind thiol groups on aptamers. Five microliters of aptamer at increasing concentration were mixed to each 50 µl gel.

Chitosan is one of the most investigated natural polymers for TE applications, because it is highly biocompatible [146]. However, some cell types grow slowly on chitosan films, and consequently they were chosen as substrates to be enriched with aptamers. Aptamers were immobilized on 2% chitosan films ($r = 3.0$ mm; $h = 0.25$ mm) at increasing concentration by exploiting the spontaneous ability of chitosan to bind sulfur-containing substances [147].
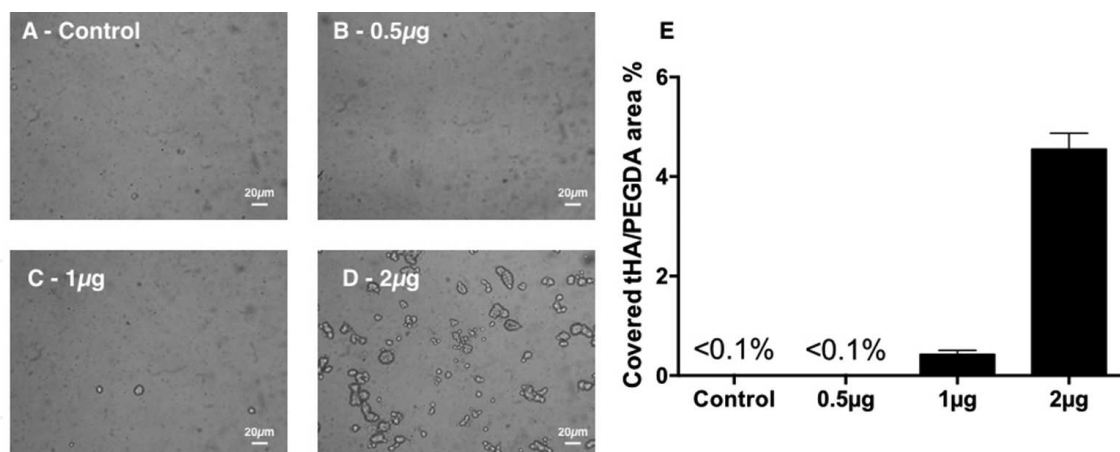

Figure 4. Study of aptamer ability to enhance the proliferation of human osteoblasts (hOB) on tHA/PEGDA hydrogel. Microphotographs, taken with an inverted microscope, showing hOB cells on tHA/PEGDA after 48 h of culture (A–D). The rate of cell growth is proportional to the quantity of aptamer used for the functionalization (E).

Five thousand hOB cells (human osteoblasts) on tHA/PEGDA gels and 5000 MC3T3-E1 cells (murine preosteoblasts from bone/calvaria) on 2% chitosan films were cultured for 7 days. Cells were monitored day by day with an inverted microscope.

Cell proliferation on tHA/PEGDA and chitosan substrates is shown in **Figures 4** and **5**.
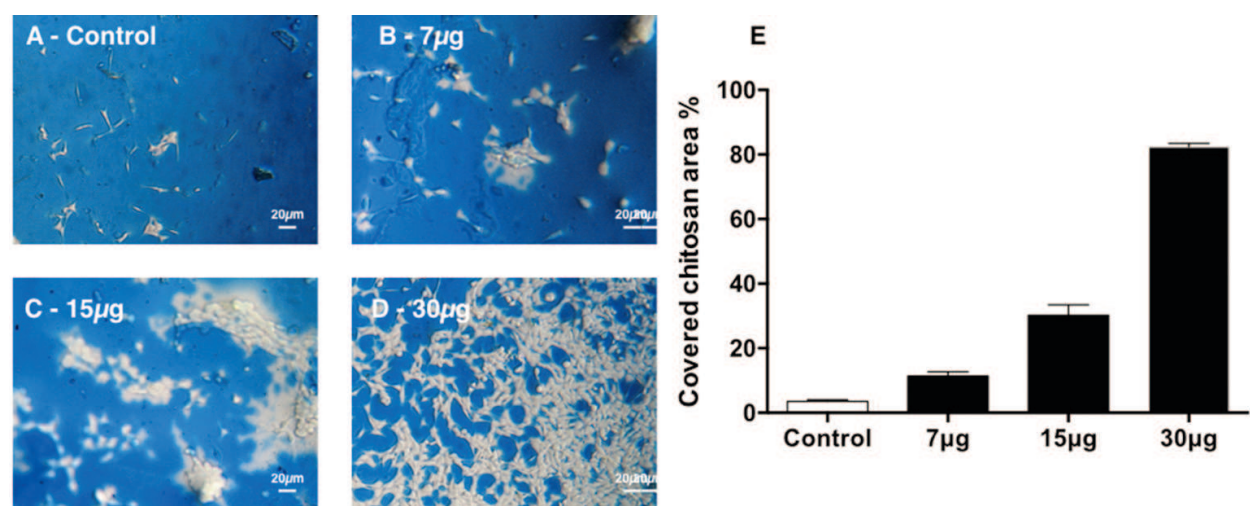
Figure 5. Study of aptamer ability to enhance the proliferation of murine osteoblastic cells (MC3T3-E1) on 2% chitosan films. Microphotographs, taken with an inverted microscope, showing MC3T3-E1 cells on 2% chitosan films after 48 h of culture and stained with the Trypan Blue to discriminate viable and dead cells. The rate of cell growth is proportional to the quantity of aptamer used for the functionalization (E).

In both the cases, aptamers increase the number of adhering cells and the rate of cell growth is proportional to the amount of aptamer used. Cell morphology appear round both in control groups and in aptamer-rich samples, unlike the flattened spindle shape morphology that is normally observed on tissue culture plastic substrates, and is routinely associated to firm cell adhesion. Although cell adhesion does appear improved in the presence of aptamers, as indicated by a significantly higher number of cells, the culture substrate is mechanically elastic and the normal morphological features of a good adhesion cannot be achieved.
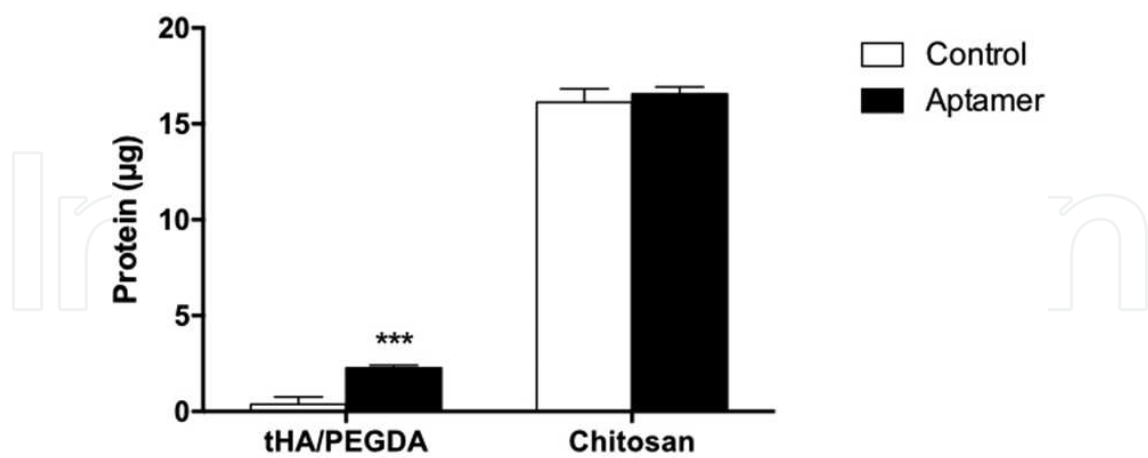

Figure 6. Histograms representing the amount of protein adsorbed on polymeric scaffolds with or without aptamers. Scaffolds were incubated for 2 h with 30 μg of proteins. The amount of protein bind by the scaffold was quantitated through the Bradford.

Although aptamers act on both substrates presumably in comparable ways, by binding fibronectin, the rationale for their use is possibly different, as suggested by results of protein

adsorption assays reported in **Figure 6**, where 30 μg of serum protein were incubated for 2 h on different scaffolds with or without aptamers and quantitated with Bradford.

The presence of aptamers on tHA/PEGDA quantitatively increases the amount of fibronectin on the gel and this may explain the improved cell adhesion and proliferation. Cells on control gels do not get in contact with adsorbed proteins and lack good attachment points for their integrins. Availability of a higher amount of adhesive protein then results in more firmly adhering cells. On the other hand, chitosan is known to bind massive amounts of protein from the supernatant and aptamers do not affect the quantity of adsorbed proteins. A viable hypothesis for the effects of aptamers on chitosan is therefore that aptamers may affect the quality of adsorbed protein. Aptamers may preserve the natural conformation of fibronectin on films, without unfolding it and maintaining a favorable exposure of adhesion sequences for cells.

Evidences reported show that aptamers are a viable approach to improve the biocompatibility of scaffolds, ameliorating the process of adhesive protein adsorption on surfaces both quantitatively and qualitatively, and should further investigated to create tissue-specific scaffold for tissue engineering.

5. Summary

Scaffolds for tissue engineering should support an appropriate cellular activity. In particular, cell adhesion and proliferation depend mainly on the efficiency of protein adsorption at the interface, a process deeply influenced by surface chemistry. Nowadays, a wide number of treatments have been proposed to enhance scaffold biocompatibility, including physical and chemical treatments or biological coatings. In this chapter we reported on the use of aptamers to improve scaffold biocompatibility.

After a general presentation on tissue engineering in Section 1, Section 2 described the rationale to control protein adsorption on biomaterial surfaces. A panoramic view of the methods developed and reported in literature to improve scaffold biocompatibility was reviewed. At the end of the section the possibility of using aptamers for this goal was outlined.

Section 3 contained general information about aptamers. The technique to obtain aptamers (SELEX) was well described and a general view on the use of aptamers in biomedical applications was outlined. Finally, in Section 4 after the explanation of the rationale to use aptamers as enhancers for scaffold biocompatibility, our preliminary results were reported. In particular, we investigated the possibility to immobilize aptamers on different substrates to improve scaffold biocompatibility *in vitro*, with similar results. Aptamers were bound to tHA/PEGDA hydrogels or to chitosan films: in both the cases the adsorption of proteins was ameliorated, as well as the adhesion and proliferation of cells. The results obtained paved the way to further investigation of the use of aptamers in combination with scaffolds for tissue engineering applications.

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Conflict of interest

The authors have no conflict of interest to disclose.

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References

[1] Mason, C. and P. Dunnill, A brief definition of regenerative medicine. Regen Med, 2008. 3(1): p. 1–5.

[2] Langer, R. and J.P. Vacanti, Tissue engineering. Science, 1993. 260(5110): p. 920–926.

[3] Dimitriou, R., et al., Bone regeneration: current concepts and future directions. BMC Med, 2011. 9: p. 66.

[4] Oryan, A., et al., Bone regenerative medicine: classic options, novel strategies, and future directions. J Orthop Surg Res, 2014. 9(1): p. 18.

[5] Giannoudis, P.V., T.A. Einhorn, and D. Marsh, Fracture healing: the diamond concept. Injury, 2007. 38 Suppl 4: p. S3–S6.

[6] Albrektsson, T. and C. Johansson, Osteoinduction, osteoconduction and osseointegration. Eur Spine J, 2001. 10 Suppl 2: p. S96–S101.

[7] Dimitriou, R., E. Tsiridis, and P.V. Giannoudis, Current concepts of molecular aspects of bone healing. Injury, 2005. 36(12): p. 1392–1404.

[8] Pountos, I., et al., Efficacy of minimally invasive techniques for enhancement of fracture healing: evidence today. Int Orthop, 2010. 34(1): p. 3–12.

[9] Matsumoto, T., et al., Therapeutic potential of vasculogenesis and osteogenesis promoted by peripheral blood CD34-positive cells for functional bone healing. Am J Pathol, 2006. 169(4): p. 1440–1457.

[10] Im, G.I., Y.W. Shin, and K.B. Lee, Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? Osteoarthritis Cartilage, 2005. 13(10): p. 845–853.

[11] Niemeyer, P., et al., Comparison of mesenchymal stem cells from bone marrow and adipose tissue for bone regeneration in a critical size defect of the sheep tibia and the influence of platelet-rich plasma. Biomaterials, 2010. 31(13): p. 3572–3579.

[12] Seo, B.M., et al., Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet, 2004. 364(9429): p. 149–155.

[13] Seo, B.M., et al., Recovery of stem cells from cryopreserved periodontal ligament. J Dent Res, 2005. 84(10): p. 907–912.

[14] Egusa, H., et al., Stem cells in dentistry – part I: stem cell sources. J Prosthodont Res, 2012. 56(3): p. 151–165.

[15] Finkemeier, C.G., Bone-grafting and bone-graft substitutes. J Bone Joint Surg Am, 2002. 84-A(3): p. 454–464.

[16] Giannoudis, P.V., H. Dinopoulos, and E. Tsiridis, Bone substitutes: an update. Injury, 2005. 36 Suppl 3: p. S20–S27.

[17] Rose, F.R. and R.O. Oreffo, Bone tissue engineering: hope vs hype. Biochem Biophys Res Commun, 2002. 292(1): p. 1–7.

[18] Salgado, A.J., O.P. Coutinho, and R.L. Reis, Bone tissue engineering: state of the art and future trends. Macromol Biosci, 2004. 4(8): p. 743–765.

[19] Kokemueller, H., et al., Prefabrication of vascularized bioartificial bone grafts in vivo for segmental mandibular reconstruction: experimental pilot study in sheep and first clinical application. Int J Oral Maxillofac Surg, 2010. 39(4): p. 379–387.

[20] Ohgushi, H., et al., Tissue engineered ceramic artificial joint – ex vivo osteogenic differentiation of patient mesenchymal cells on total ankle joints for treatment of osteoarthritis. Biomaterials, 2005. 26(22): p. 4654–4661.

[21] Kim, S.J., et al., A multi-center, randomized, clinical study to compare the effect and safety of autologous cultured osteoblast (Ossron) injection to treat fractures. BMC Musculoskelet Disord, 2009. 10: p. 20.

[22] Langer, R. and D.A. Tirrell, Designing materials for biology and medicine. Nature, 2004. 428(6982): p. 487–492.

[23] Dhandayuthapani, S., et al., Bromelain-induced apoptosis in GI-101A breast cancer cells. J Med Food, 2012. 15(4): p. 344–349.

[24] Kikuchi, M., Hydroxyapatite/collagen bone-like nanocomposite. Biol Pharm Bull, 2013. 36(11): p. 1666–1669.

[25] Lee, S.S., et al., Bone regeneration with low dose BMP-2 amplified by biomimetic supramolecular nanofibers within collagen scaffolds. Biomaterials, 2013. 34(2): p. 452–459.

[26] Pastorino, L., et al., Oriented collagen nanocoatings for tissue engineering. Colloids Surf B Biointerfaces, 2014. 114: p. 372–378.

[27] Nishiyama, Y., P. Langan, and H. Chanzy, Crystal structure and hydrogen-bonding system in cellulose Ibeta from synchrotron X-ray and neutron fiber diffraction. J Am Chem Soc, 2002. 124(31): p. 9074–9082.

[28] Agnihotri, S.A., N.N. Mallikarjuna, and T.M. Aminabhavi, Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J Control Release, 2004. 100(1): p. 5–28.

[29] Aravamudhan, A., et al., Cellulose and collagen derived micro-nano structured scaffolds for bone tissue engineering. J Biomed Nanotechnol, 2013. 9(4): p. 719–731.

[30] Perez, R.A., et al., Utilizing core-shell fibrous collagen-alginate hydrogel cell delivery system for bone tissue engineering. Tissue Eng Part A, 2014. 20(1–2): p. 103–114.

[31] Mistura, D.V., et al., Development, characterization, and cellular adhesion of poly(L-lactic acid)/poly(caprolactone triol) membranes for potential application in bone tissue regeneration. Artif Organs, 2013. 37(11): p. 978–984.

[32] Perez, R.A. and M.P. Ginebra, Injectable collagen/alpha-tricalcium phosphate cement: collagen-mineral phase interactions and cell response. J Mater Sci Mater Med, 2013. 24(2): p. 381–393.

[33] Williams, D.F., On the mechanisms of biocompatibility. Biomaterials, 2008. 29(20): p. 2941–2953.

[34] Donaruma, L.G., Definitions in biomaterials, D. F. Williams, Ed., Elsevier, Amsterdam, 1987, 72 pp. J Polym Sci Part C: Polym Lett, 1988. 26(9): p. 414.

[35] Williams, D.F., The Williams dictionary of biomaterials, L.U. Press. 1999.

[36] A., M., Dinamiche delle interazioni materiale-ambiente biologico, in Biomateriali – dagli impianti protesici alla medicina rigenerativa, Patròn Editore, editor, Bologna, 2005. p. 27–47.

[37] Tang, L. and W. Hu, Molecular determinants of biocompatibility. Expert Rev Med Devices, 2005. 2(4): p. 493–500.

[38] Thevenot, P., et al., Surface chemistry influences cancer killing effect of TiO_2 nanoparticles. Nanomedicine, 2008. 4(3): p. 226–236.

[39] Tegoulia, V.A., et al., Surface properties, fibrinogen adsorption, and cellular interactions of a novel phosphorylcholine-containing self-assembled monolayer on gold. Langmuir, 2001. 17(14): p. 4396–4404.

[40] Martins, M.C., B.D. Ratner, and M.A. Barbosa, Protein adsorption on mixtures of hydroxyl- and methyl-terminated alkanethiols self-assembled monolayers. J Biomed Mater Res A, 2003. 67(1): p. 158–171.

[41] Agashe, M., et al., Molecular simulation to characterize the adsorption behavior of a fibrinogen gamma-chain fragment. Langmuir, 2005. 21(3): p. 1103–1117.

[42] Ruckenstein, E. and Z.F. Li, Surface modification and functionalization through the self-assembled monolayer and graft polymerization. Adv Colloid Interface Sci, 2005. 113(1): p. 43–63.

[43] Tang, L., L. Liu, and H.B. Elwing, Complement activation and inflammation triggered by model biomaterial surfaces. J Biomed Mater Res, 1998. 41(2): p. 333–340.

[44] Barbosa, J.N., et al., The influence of functional groups of self-assembled monolayers on fibrous capsule formation and cell recruitment. J Biomed Mater Res A, 2006. 76(4): p. 737–743.

[45] Dyshlovenko, S., et al., Pulsed laser modification of plasma-sprayed coatings: experimental processing of hydroxyapatite and numerical simulation. Surface Coat Technol, 2006. 201(6): p. 2248–2255.

[46] Forster, Y., et al., Surface modification of implants in long bone. Biomatter, 2012. 2(3): p. 149–157.

[47] Rezania, A., et al., The detachment strength and morphology of bone cells contacting materials modified with a peptide sequence found within bone sialoprotein. J Biomed Mater Res, 1997. 37(1): p. 9–19.

[48] Dee, K.C., T.T. Anderson, and R. Bizios, Osteoblast population migration characteristics on substrates modified with immobilized adhesive peptides. Biomaterials, 1999. 20(3): p. 221–227.

[49] Zreiqat, H., et al., Differentiation of human bone-derived cells grown on GRGDSP-peptide bound titanium surfaces. J Biomed Mater Res A, 2003. 64(1): p. 105–113.

[50] Ferris, D.M., et al., RGD-coated titanium implants stimulate increased bone formation in vivo. Biomaterials, 1999. 20(23–24): p. 2323–2331.

[51] Elmengaard, B., J.E. Bechtold, and K. Soballe, In vivo effects of RGD-coated titanium implants inserted in two bone-gap models. J Biomed Mater Res A, 2005. 75(2): p. 249–255.

[52] Rammelt, S., et al., Coating of titanium implants with collagen, RGD peptide and chondroitin sulfate. Biomaterials, 2006. 27(32): p. 5561–5571.

[53] Hennessy, K.M., et al., The effect of RGD peptides on osseointegration of hydroxyapatite biomaterials. Biomaterials, 2008. 29(21): p. 3075–3083.

[54] Geissler, U., et al., Collagen type I-coating of Ti6Al4V promotes adhesion of osteoblasts. J Biomed Mater Res, 2000. 51(4): p. 752–760.

[55] Roehlecke, C., et al., Synergistic effect of titanium alloy and collagen type I on cell adhesion, proliferation and differentiation of osteoblast-like cells. Cells Tissues Organs, 2001. 168(3): p. 178–187.

[56] Huang, L., et al., The effect of hyaluronan on osteoblast proliferation and differentiation in rat calvarial-derived cell cultures. J Biomed Mater Res A, 2003. 66(4): p. 880–884.

[57] Takada, T., et al., Sulfated polysaccharides enhance the biological activities of bone morphogenetic proteins. J Biol Chem, 2003. 278(44): p. 43229–43235.

[58] Song, K.M., S. Lee, and C. Ban, Aptamers and their biological applications. Sensors (Basel), 2012. 12(1): p. 612–631.

[59] Ellington, A.D. and J.W. Szostak, In vitro selection of RNA molecules that bind specific ligands. Nature, 1990. 346(6287): p. 818–822.

[60] Mascini, M., I. Palchetti, and S. Tombelli, Nucleic acid and peptide aptamers: fundamentals and bioanalytical aspects. Angew Chem Int Ed Engl, 2012. 51(6): p. 1316–1332.

[61] Ku, T.H., et al., Nucleic acid aptamers: an emerging tool for biotechnology and biomedical sensing. Sensors (Basel), 2015. 15(7): p. 16281–16313.

[62] Sun, H. and Y. Zu, A highlight of recent advances in aptamer technology and its application. Molecules, 2015. 20(7): p. 11959–11980.

[63] Geiger, A., et al., RNA aptamers that bind L-arginine with sub-micromolar dissociation constants and high enantioselectivity. Nucleic Acids Res, 1996. 24(6): p. 1029–1036.

[64] Eyetech Study, G., Preclinical and phase 1A clinical evaluation of an anti-VEGF pegylated aptamer (EYE001) for the treatment of exudative age-related macular degeneration. Retina, 2002. 22(2): p. 143–152.

[65] Eyetech Study, G., Anti-vascular endothelial growth factor therapy for subfoveal choroidal neovascularization secondary to age-related macular degeneration: phase II study results. Ophthalmology, 2003. 110(5): p. 979–986.

[66] Ireson, C.R. and L.R. Kelland, Discovery and development of anticancer aptamers. Mol Cancer Ther, 2006. 5(12): p. 2957–2962.

[67] Xiang, D., et al., Nucleic acid aptamer-guided cancer therapeutics and diagnostics: the next generation of cancer medicine. Theranostics, 2015. 5(1): p. 23–42.

[68] Jayasena, S.D., Aptamers: an emerging class of molecules that rival antibodies in diagnostics. Clin Chem, 1999. 45(9): p. 1628–1650.

[69] Ng, E.W. and A.P. Adamis, Anti-VEGF aptamer (pegaptanib) therapy for ocular vascular diseases. Ann N Y Acad Sci, 2006. 1082: p. 151–171.

[70] Nucleic acid aptamers for diagnostics and therapeutics: global markets. Available from: http://www.marketresearch.com/BCC-Research-v374/Nucleic-Acid-Aptamers-Diagnostics-Therapeutics-8412378/.

[71] Tuerk, C. and L. Gold, Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science, 1990. 249(4968): p. 505–510.

[72] Kulbachinskiy, A.V., Methods for selection of aptamers to protein targets. Biochemistry (Mosc), 2007. 72(13): p. 1505–1518.

[73] Tombelli, S., M. Minunni, and M. Mascini, Analytical applications of aptamers. Biosens Bioelectron, 2005. 20(12): p. 2424–2434.

[74] Gopinath, S.C., Methods developed for SELEX. Anal Bioanal Chem, 2007. 387(1): p. 171–182.

[75] Vianini, E., M. Palumbo, and B. Gatto, In vitro selection of DNA aptamers that bind L-tyrosinamide. Bioorg Med Chem, 2001. 9(10): p. 2543–2548.

[76] Levesque, D., et al., In vitro selection and characterization of RNA aptamers binding thyroxine hormone. Biochem J, 2007. 403(1): p. 129–138.

[77] Song, K.M., et al., Gold nanoparticle-based colorimetric detection of kanamycin using a DNA aptamer. Anal Biochem, 2011. 415(2): p. 175–181.

[78] Niazi, J.H., S.J. Lee, and M.B. Gu, Single-stranded DNA aptamers specific for antibiotics tetracyclines. Bioorg Med Chem, 2008. 16(15): p. 7245–7253.

[79] Wang, A.Z., et al., Superparamagnetic iron oxide nanoparticle-aptamer bioconjugates for combined prostate cancer imaging and therapy. ChemMedChem, 2008. 3(9): p. 1311–1315.

[80] Joeng, C.B., et al., ssDNA aptamers that recognize diclofenac and 2-anilinophenylace-tic acid. Bioorg Med Chem, 2009. 17(15): p. 5380–5387.

[81] Mendonsa, S.D. and M.T. Bowser, In vitro selection of high-affinity DNA ligands for human IgE using capillary electrophoresis. Anal Chem, 2004. 76(18): p. 5387–5392.

[82] Mendonsa, S.D. and M.T. Bowser, In vitro selection of aptamers with affinity for neuropeptide Y using capillary electrophoresis. J Am Chem Soc, 2005. 127(26): p. 9382–9383.

[83] Kunii, T., et al., Selection of DNA aptamers recognizing small cell lung cancer using living cell-SELEX. Analyst, 2011. 136(7): p. 1310–1312.

[84] Van Simaeys, D., et al., Study of the molecular recognition of aptamers selected through ovarian cancer cell-SELEX. PLoS One, 2010. 5(11): p. e13770.

[85] Daniels, D.A., et al., A tenascin-C aptamer identified by tumor cell SELEX: systematic evolution of ligands by exponential enrichment. Proc Natl Acad Sci U S A, 2003. 100(26): p. 15416–15421.

[86] Gold, L., et al., Diversity of oligonucleotide functions. Annu Rev Biochem, 1995. 64: p. 763–797.

[87] Green, L.S., et al., Inhibitory DNA ligands to platelet-derived growth factor B-chain. Biochemistry, 1996. 35(45): p. 14413–14424.

[88] Zhou, J., et al., Structural prediction and binding analysis of hybridized aptamers. J Mol Recognit, 2011. 24(1): p. 119–126.

[89] Shigdar, S., et al., RNA aptamers targeting cancer stem cell marker CD133. Cancer Lett, 2013. 330(1): p. 84–95.

[90] Qiao, L., et al., A new application of aptamer: one-step purification and immobiliza-tion of enzyme from cell lysates for biocatalysis. J Biotechnol, 2015. 203: p. 68–76.

[91] Saberian-Borujeni, M., et al., Nanoscaled aptasensors for multi-analyte sensing. Bioimpacts, 2014. 4(4): p. 205–215.

[92] Chen, A. and S. Yang, Replacing antibodies with aptamers in lateral flow immunoas-say. Biosens Bioelectron, 2015. 71: p. 230–242.

[93] Wu, J., et al., Recent trends in SELEX technique and its application to food safety monitoring. Mikrochim Acta, 2014. 181(5–6): p. 479–491.

[94] Hayat, A. and J.L. Marty, Aptamer based electrochemical sensors for emerging environmental pollutants. Front Chem, 2014. 2 41 doi: 10.3389/fchem.2014.00041.

[95] Lee, J.H., F. Jucker, and A. Pardi, Imino proton exchange rates imply an induced-fit binding mechanism for the VEGF165-targeting aptamer, Macugen. FEBS Lett, 2008. 582(13): p. 1835–1839.

[96]   Inc, E., Phase 3 study presented at world ophthalmology congress evaluates investigational use of macugen in patients with diabetic macular edema. 2010.

[97]   Sultan, M.B., et al., A phase 2/3, multicenter, randomized, double-masked, 2-year trial of pegaptanib sodium for the treatment of diabetic macular edema. Ophthalmology, 2011. 118(6): p. 1107–1118.

[98]   Hermann Eye Center, WV. A Pilot Study for the Treatment of Iris Neovascularization With Macugen. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2006 . Available from: https://clinicaltrials.gov/ct2/show/NCT00295828 NLM Identifier: NCT00295828.

[99]   Rosenbaum JT. Future for biological therapy for uveitis. Curr Opin Ophthalmol. 2010 Nov;21(6):473–7.

[100]   Pfizer. Long-Term Use Of Pegaptanib In Patients For Subfoveal Choroidal Neovascularization Secondary To Age-Related Macular Degeneration In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2008- . Available from: https://clinicaltrials.gov/ct2/show/NCT00845273 NLM Identifier: NCT00845273.

[101]   Jo, N., et al., Inhibition of platelet-derived growth factor B signaling enhances the efficacy of anti-vascular endothelial growth factor therapy in multiple models of ocular neovascularization. Am J Pathol, 2006. 168(6): p. 2036–2053.

[102]   Di Cera, E., Thrombin as procoagulant and anticoagulant. J Thromb Haemost, 2007. 5 Suppl 1: p. 196–202.

[103]   Bock, L.C., et al., Selection of single-stranded DNA molecules that bind and inhibit human thrombin. Nature, 1992. 355(6360): p. 564–566.

[104]   Griffin, L.C., et al., In vivo anticoagulant properties of a novel nucleotide-based thrombin inhibitor and demonstration of regional anticoagulation in extracorporeal circuits. Blood, 1993. 81(12): p. 3271–3276.

[105]   ARCA Biopharma. Study of NU172 as Anticoagulation in Patients Undergoing Off-pump CABG Surgery (SNAP-CABG-OFF). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2008- . Available from: https://clinicaltrials.gov/ct2/show/NCT00808964 NLM Identifier: NCT00808964.

[106]   Bates, P.J., et al., Discovery and development of the G-rich oligonucleotide AS1411 as a novel treatment for cancer. Exp Mol Pathol, 2009. 86(3): p. 151–164.

[107]   Girvan, A.C., et al., AGRO100 inhibits activation of nuclear factor-kappaB (NF-kappaB) by forming a complex with NF-kappaB essential modulator (NEMO) and nucleolin. Mol Cancer Ther, 2006. 5(7): p. 1790–1799.

[108]   Soundararajan, S., et al., The nucleolin targeting aptamer AS1411 destabilizes Bcl-2 messenger RNA in human breast cancer cells. Cancer Res, 2008. 68(7): p. 2358–2365.

[109] Bates, P.J., et al., Antiproliferative activity of G-rich oligonucleotides correlates with protein binding. J Biol Chem, 1999. 274(37): p. 26369–26377.

[110] Bruno, J.G., Predicting the uncertain future of aptamer-based diagnostics and therapeutics. Molecules, 2015. 20(4): p. 6866–6887.

[111] Duclair, S., et al., High-affinity RNA aptamers against the HIV-1 protease inhibit both in vitro protease activity and late events of viral replication. Mol Ther Nucl Acids, 2015. 4: p. e228.

[112] Cheng, C., et al., Potent inhibition of human influenza H5N1 virus by oligonucleotides derived by SELEX. Biochem Biophys Res Commun, 2008. 366(3): p. 670–674.

[113] Binning, J.M., et al., Development of RNA aptamers targeting Ebola virus VP35. Biochemistry, 2013. 52(47): p. 8406–8419.

[114] Shum, K.T., J. Zhou, and J.J. Rossi, Aptamer-based therapeutics: new approaches to combat human viral diseases. Pharmaceuticals (Basel), 2013. 6(12): p. 1507–1542.

[115] Wandtke, T., J. Wozniak, and P. Kopinski, Aptamers in diagnostics and treatment of viral infections. Viruses, 2015. 7(2): p. 751–780.

[116] Gopinath, S.C. and P.K. Kumar, Aptamers that bind to the hemagglutinin of the recent pandemic influenza virus H1N1 and efficiently inhibit agglutination. Acta Biomater, 2013. 9(11): p. 8932–8941.

[117] Kwon, H.M., et al., An RNA aptamer that specifically binds to the glycosylated hemagglutinin of avian influenza virus and suppresses viral infection in cells. PLoS One, 2014. 9(5): p. e97574.

[118] Musafia, B., R. Oren-Banaroya, and S. Noiman, Designing anti-influenza aptamers: novel quantitative structure activity relationship approach gives insights into aptamer-virus interaction. PLoS One, 2014. 9(5): p. e97696.

[119] Shiratori, I., et al., Selection of DNA aptamers that bind to influenza A viruses with high affinity and broad subtype specificity. Biochem Biophys Res Commun, 2014. 443(1): p. 37–41.

[120] Suenaga, E. and P.K. Kumar, An aptamer that binds efficiently to the hemagglutinins of highly pathogenic avian influenza viruses (H5N1 and H7N7) and inhibits hemagglutinin-glycan interactions. Acta Biomater, 2014. 10(3): p. 1314–1323.

[121] Bruno, J.G., et al., Development of DNA aptamers for cytochemical detection of acetylcholine. In Vitro Cell Dev Biol Anim, 2008. 44(3–4): p. 63–72.

[122] Bruno, J.G., et al., A novel screening method for competitive FRET-aptamers applied to *E. coli* assay development. J Fluoresc, 2010. 20(6): p. 1211–1223.

[123] Schlesinger, S.R., et al., Metallo-β-lactamases and aptamer-based inhibition. Pharmaceuticals 2011, 4, 419–428 doi: 10.3390/ph4020419

[124] Stecker, J.R., et al., Dynamics and visualization of MCF7 adenocarcinoma cell death by aptamer-C1q-mediated membrane attack. Nucleic Acid Ther, 2012. 22(4): p. 275–282.

[125] Shangguan, D., et al., Cell-specific aptamer probes for membrane protein elucidation in cancer cells. J Proteome Res, 2008. 7(5): p. 2133–2139.

[126] Mallikaratchy, P., et al., Aptamer directly evolved from live cells recognizes membrane bound immunoglobin heavy mu chain in Burkitt's lymphoma cells. Mol Cell Proteomics, 2007. 6(12): p. 2230–2238.

[127] Van Simaeys, D., et al., Identification of cell membrane protein stress-induced phosphoprotein 1 as a potential ovarian cancer biomarker using aptamers selected by cell systematic evolution of ligands by exponential enrichment. Anal Chem, 2014. 86(9): p. 4521–4527.

[128] Hamaguchi, N., A. Ellington, and M. Stanton, Aptamer beacons for the direct detection of proteins. Anal Biochem, 2001. 294(2): p. 126–131.

[129] Li, J.J., X. Fang, and W. Tan, Molecular aptamer beacons for real-time protein recognition. Biochem Biophys Res Commun, 2002. 292(1): p. 31–40.

[130] Levy, M., S.F. Cater, and A.D. Ellington, Quantum-dot aptamer beacons for the detection of proteins. Chembiochem, 2005. 6(12): p. 2163–2166.

[131] Bagalkot, V., et al., Quantum dot-aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer. Nano Lett, 2007. 7(10): p. 3065–3070.

[132] Zhou, D., Quantum dot-nucleic acid/aptamer bioconjugate-based fluorimetric biosensors. Biochem Soc Trans, 2012. 40(4): p. 635–639.

[133] Lee, K.Y., et al., Bioimaging of nucleolin aptamer-containing 5-(N-benzylcarboxyamide)-2′-deoxyuridine more capable of specific binding to targets in cancer cells. J Biomed Biotechnol, 2010. 2010: p. 168306.

[134] Min, K., et al., Simultaneous electrochemical detection of both PSMA (+) and PSMA (–) prostate cancer cells using an RNA/peptide dual-aptamer probe. Chem Commun (Camb), 2010. 46(30): p. 5566–5568.

[135] Kim, G.D., et al., 6″-Debromohamacanthin A, a bis (indole) alkaloid, inhibits angiogenesis by targeting the VEGFR2-mediated PI3K/AKT/mTOR signaling pathways. Mar Drugs, 2013. 11(4): p. 1087–1103.

[136] Min, K., et al., Dual-aptamer-based delivery vehicle of doxorubicin to both PSMA (+) and PSMA (–) prostate cancers. Biomaterials, 2011. 32(8): p. 2124–2132.

[137] Chu, T.C., et al., Aptamer mediated siRNA delivery. Nucl Acids Res, 2006. 34(10): p. e73.

[138] Farokhzad, O.C., et al., Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. Cancer Res, 2004. 64(21): p. 7668–7672.

[139] Farokhzad, O.C., et al., Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. Proc Natl Acad Sci U S A, 2006. 103(16): p. 6315–6320.

[140] Guo, J., et al., Aptamer-functionalized PEG-PLGA nanoparticles for enhanced anti-glioma drug delivery. Biomaterials, 2011. 32(31): p. 8010–8020.

[141] Lee, I.H., et al., Targeted chemoimmunotherapy using drug-loaded aptamer-dendrimer bioconjugates. J Control Release, 2011. 155(3): p. 435–441.

[142] Cao, Z., et al., Reversible cell-specific drug delivery with aptamer-functionalized liposomes. Angew Chem Int Ed Engl, 2009. 48(35): p. 6494–6498.

[143] Wu, J., et al., Nucleolin targeting AS1411 modified protein nanoparticle for antitumor drugs delivery. Mol Pharm, 2013. 10(10): p. 3555–3563.

[144] Hoffmann, J., et al., Immobilized DNA aptamers used as potent attractors for porcine endothelial precursor cells. J Biomed Mater Res A, 2008. 84(3): p. 614–621.

[145] Chen, N., et al., Cell adhesion on an artificial extracellular matrix using aptamer-functionalized PEG hydrogels. Biomaterials, 2012. 33(5): p. 1353–1362.

[146] Gasperini, L., J.F. Mano, and R.L. Reis, Natural polymers for the microencapsulation of cells. J R Soc Interface, 2014. 11(100): p. 20140817.

[147] Elviri, L., et al., Macroporous chitosan hydrogels: effects of sulfur on the loading and release behaviour of amino acid-based compounds. Carbohydr Polym, 2015. 132: p. 50–58.