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REVIEW

Aptamers improve the bioactivity of biomaterials

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ABSTRACT

The research in the field of biomaterials has largely focused on the development of methods to enhance their bioactivity. Adopted strategies include chemical-physical approaches, but mainly the use of monoclonal antibodies, which confer the system target specificity. However, antibodies are marred by numerous issues, such as low physical-chemical stability or the risk of inducing immunological responses in the host, which often make their use difficult. Aptamers – a new class of molecules discovered in the early nineties – are small oligonucleotides, or in some cases peptides, and have been proposed to rival antibodies in biomedicine, countering at least some of the antibody-related drawbacks. The aim of this review is to provide a background to nucleic acid aptamers and to explore their novel applications. In addition to providing brief overview of their therapeutics applications, here we have assessed the methods that employ aptamers to improve the bioactivity of biomaterials, in particular, those that enhance targeting properties of drug delivery systems for chemotherapy, and those ameliorating scaffold biocompatibility for tissue engineering approaches.

KEYWORDS: Nucleic acid aptamers, biomaterials, bioactivity, drug delivery system, scaffold

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BIOMATERIALS AND THEIR BIOCOMPATIBILITY

The term biomaterial was first defined in 1987 at the Consensus Conference on the Definitions in Biomaterial Science of the European Society for Biomaterials (Williams, 1987), as *"non viable material used in a medical device, intended to interact with biological systems"*. Subsequently, with the advancements in cell and molecular biology, chemistry, material science and engineering, the term has significantly evolved over the past 30 years (Keane and Badylak, 2014), and biomaterials are nowadays defined as *"materials intended to interface with biological systems to evaluate, treat, argument or replace any tissue or function of the body"*.

A concept closely connected to that of biomaterial is biocompatibility. Materials were first considered "biomaterials", and therefore are biocompatible, if they could be placed in contact with tissues without damaging them, thus being essentially inert. However, research progressively revealed that biological inertia is impossible to achieve and that any material that comes in contact with a tissue induces a non-self response from the host immune system. The term biocompatibility was then revised and for years associated with the lack of toxicity, immunogenicity, tumorigenicity and irritancy against the human body. Subsequently, new evidence in the early eighties led to an updated definition of biocompatibility as it became clear that all materials react with tissues and are not inert; it was also shown that biological responses to a biomaterial are different among tissues and that tissues themselves affect material biocompatibility. Furthermore, clinical evidence indicated that some situations require materials to get degraded and removed from the host after accomplishing their function (Williams, 1987). Taking all these considerations together, at the Consensus Conferences in Boston in 1987, the definition of biocompatibility was outlined as follows: *"Biocompatibility refers to the ability of a material to perform with an appropriate host response in a specific situation"* (Williams, 1987). This definition of biocompatibility led to a new concept of bioactivity, which can be associated to the ability of the material to perform for its function.

The aim of this review is to focus on the concept of bioactivity related to biomaterials used as drug delivery systems (DDS) or as scaffold for tissue engineering (TE) applications and on how to improve aptamers performance and thus their bioactivity.

Biomaterials as drug delivery system in chemotherapy

Cancer is currently the second cause of death worldwide. It is a complex phenomenon and many factors affect its outbreak and diffusion, making its therapy one of the most ambitious aims in the field of biomedical research. Different strategies have been developed for its treatment, including surgical removal, hyperthermia, immunotherapy, targeted therapy, radiotherapy, hormone therapy, stem cell therapy and chemotherapy (Ravichandran and Mandhari, 2015; Assi et al, 2016; Hellmann et al, 2016; Pederson et al, 2016; Qiao et al, 2016). With particular regard to chemotherapy, it involves the use of anti-cancer drugs against fast dividing cells, which often leads to adverse effects on the healthy fast dividing cells of the organisms, not just on the cancerous ones. Moreover, in the long run cancer cells become resistant to these chemicals and adverse

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effects may exceed the therapeutic ones, worsening the therapeutic index of drugs already characterized by a close vicinity of effective and toxic dose (van Elk et al, 2016).

To exceed these adverse effects, in recent years nanotechnology has played a pivotal role in chemotherapy for cancer treatment, through the conjugation of different biomaterial-based nano vectors such as liposomes, micelles, dendrimers, synthetic or polymeric nanostructures, with these drugs (Vahed et al, in press). In particular, liposomes and micelles seem to be a very effective option in DDS for chemotherapy and several formulations have been approved or are under clinical trials for the treatment of cancer, as reported in Table 1 and 2. The efficacy of these methods have always depended on their passive accumulation in the tumours via the permeability and retention effect (EPR) (van Elk et al, 2016). However, the evidence that the EPR effect is very heterogeneous and that it is different between tumour types and from patient to patient makes necessary the development of active targeting (bioactivity) strategies in order to ameliorate therapy specificity (Jain and Stylianopoulos T, 2010; Lammers et al, 2012). Active targeting consists in enhancing the delivery of the drug-biomaterial complex to the tumour, and to this purpose two strategies have been developed until now: (i) one based on the development of physicochemical responsive systems, and (ii) one based on the manipulation of nano vector surface with ligands able to bind over expressed molecules on cancer cells (Vahed et al, in press).

Physical-chemical responsive systems exploit the unique physicochemical characteristics of the tumours, as stimuli to trigger the release of the drug from the DDS in the specific site (van Elk et al, 2016). For example, the lower pH (pH~6.8) and the higher temperature gradient (~40°C) of tumours are often used to control responsive systems.

On the other hand, targeted systems exploit over-expressed surface molecules of cancer cells to obtain active targeted DDS by enriching them with ligands able to bind these selective molecules (Allen, 2002). Alternatively, to the use of target-ligands systems, monoclonal antibodies can be used too. For example, immunoliposomes have been designed through the immobilization of monoclonal antibodies on liposome surface to target drugs to the cells that specifically expressed the selected epitope.

Biomaterials as scaffolds for tissue engineering approaches

Regenerative medicine (RM) is a new therapeutic approach, which aims to restore structure and function of damaged tissue and organs to find a solution to that permanently damaged and untreatable (Mason and Dunnil, 2008). Tissue regeneration is a complex task and may be achieved with RM through three type of approaches: molecular, cellular and TE.

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 science towards the development of biological substitutes that restore, maintain and improve tissue function"*. TE offers great potentials in the clinical practice and is centered on the development of a scaffold, which combined with cells and molecules, allows the activation of tissue regenerative mechanisms.

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Scaffold is a central concept of TE and consists in a 3D structure designed to promote cell adhesion, proliferation and extracellular matrix (ECM) molecules deposition (Langer and Tirrell, 2004). Scaffolds can be made of biological or synthetic materials. Biological ones are derived from human, animal and vegetal tissues, while the synthetic are prepared with artificial biomaterials (Dhandayuthapani et al, 2012). Since materials of biological origin suffer from issues, such as scarce availability, safety concerns and the possibility of inflammatory and immune responses, synthetic materials are nowadays the center of increasing attention. As well as for DDS, biocompatibility is a key concept for TE approaches. A scaffold can be considered for *in vivo* application if it has been proven to be biocompatible *in vitro*, *e.g*., supporting cell adhesion and proliferation. Cell behavior heavily depends on the quality of protein adsorption at the interface, which is a spontaneous phenomenon that occurs when a material comes in contact with biological fluids (Motta, 2005). Furthermore, it has been convincingly demonstrated that shortly after the insertion of a material in an anatomical site, it is covered with a macromolecular film of host proteins which are essential for scaffold colonization from autologous cells (Tang and Hu, 2005). In this view, the physic-chemical characteristics of the material play a pivotal role during their adsorption and may conduct to their denaturation, inducing the alteration of protein conformation and consequently of their function, as well as in controlling the amount of adsorbed proteins. For that a series of methods have been developed in years to enhance scaffold surface biocompatibility, controlling the amount, the composition and the conformation of adsorbed proteins: these methods include the immobilization of short peptides or proteins on scaffolds and chemical and physical treatments.

Chemical and physical treatments exploit the ability of some proteins to bind certain chemical groups better than others, by enriching surfaces with specific functional groups through the combination of chemical and physical methods (Ruckenstein and Li, 2005; Tegoulia at al, 2001). Alternatively, the recent discovery of integrin-binding sequences opened the possibility of immobilizing them on materials and to enrich scaffolds with docking points for cells (Ruoslhati, 1996), able to enhance the adhesion, migration and differentiation of osteoblasts i*n vitro*. Consistently with this, another interesting method to improve scaffold biocompatibility concerns in their coating with entire proteins that mimic ECM, while novel approaches moved the attention to the use of antibodies as docking molecules capable to retain certain growth factors on scaffold surfaces (Geissler et al, 2000; Roehlecke, 2001; Huang et al, 2003; Takada et al, 2003; Oliveira et al, 2014).

All considered, a strategy widely used for the enhancement of material bioactivity concerns in the immobilization of monoclonal antibodies, both for DDS and for scaffolds for TE applications, and it seems also to be the most promising.

However, the use of monoclonal antibodies faces numerous issues, prompting researchers to develop new methods to enhance scaffold bioactivity. One such approach involves the use of aptamers, a new class of molecules which act in a manner similar to antibodies, but without several of their drawbacks.

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APTAMERS

In the 1980s, molecular virology studies discovered that small oligonucleotides were able to bind proteins with high affinity and specificity. This led to the use of oligonucleotides as selective receptors and to the discovery of aptamers nearly 10 years later (Song et al, 2012). In 1990, Ellington and Szostak were the first to use the term "aptamer", which derived from the fusion of the Latin *"aptus"* (to fit) and the Greek *"meros"* (part), and which identified small RNAs that bound with organic dyes (Ellington and Szostak, 1990). Nowadays, nucleic acid-based aptamers are defined as small oligonucleotides able to recognize and to bind selected target by adopting three-dimensional (3D) highly-specific conformation (Mascini et al, 2012).

Nucleic acid aptamers are short, single or double-stranded DNA or RNA oligonucleotides, 20-80 bp/6-30 kDa. Aptamer structure is constituted from a random sequence in the centre, which is important in target recognition, and is flanked by constant designed primer binding sites at the 3' and 5' ends, and necessary for aptamers amplification. The aptamer-ligand interaction is stabilized by hydrogen-bonding, van der Waal forces and from electrostatic interactions, and it is highly-specific and is able to discriminate the ligands from its analogues (*i.e.*, enantioselcetive aptamers is 12.000-fold higher affine for L-arginine than for D-arginine) (Geiger et al, 1996; Ku et al, 2015; Sun and Zu, 2015).

Due to their ability to bind several molecules, aptamers are considered an alternative to antibodies. Unlike antibodies, aptamers have low immunogenecity and low toxicity, but mainly they are not directly recognized by the human immune system as foreign agents (Eyetech Study, 2002; Eyetech Study, 2003; Ireson and Kelland, 2006). In contrast with antibodies, aptamers show a wider range of targets because they are smaller in size, can relatively easily permeate into tissue barriers and cells (Xiang ey al, 2015), and can bind with small ligands, such as ions and small molecules. Moreover, aptamer structures are thermally stable, which can withstand several cycles of denaturation/renaturation without damaging their chemical structure and consequently their binding efficiency. Finally, aptamer production and modification is cheaper, easier and faster than that of antibodies (Jayasena, 1999). In spite of their numerous advantages, aptamers have some drawbacks, such as their fast renal clearance, poor cellular uptake and intracellular degradation; however, overall, aptamer still offer a viable alternative to antibodies in several applications.

The considerable increase in the number of publications on aptamers shows that the interest in this field has continuously grown over last 25 years (Ku et al, 2015), with more than 6000 articles on aptamers indexed in the PubMed database at the end of 2016. In spite of their popularity, clinical applications of aptamers are still limited; currently, the US Food and Drug Administration (FDA) has approved only one aptamer-based drug, the Macugen®, an RNA aptamer against the vascular endothelial growth factor (VEGF) for the treatment of the wet-related macular degeneration (AMD), launched by Pfizer/Eyetech in 2004 (Ng and Adamis, 2006). Two of the key barriers in aptamers commercialization are, i) the lack of comparable response of many *in vitro* generated aptamers *in vivo*, and ii) the time-consuming and inefficient aptamer selection process.

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However, in spite of these issues, a recent market report has projected the global aptamer market to 5.4 billion of dollars by 2019.

Aptamers generation

Aptamers generation involves two steps: upstream screening and downstream screening. The first step requires the identification of full-length aptamers through the SELEX (Systematic Evolution of Ligands by EXponential Enrichment), while the second one requires the isolation of the shortest sequence able to bind with the target (Ku et al, 2015).

Upstream screening

SELEX was the technique developed *in vitro* by Ellington and Gold in 1990 to isolate specific aptamers (Ellington and Szostak, 1990; Tuerk and Gold, 1990). The process consists of three steps, which are cyclically repeated to screen sequences with the highest affinity for the target (Song, 2012). The preparation of an initial pool of oligonucleotides (library) is followed by the selection of the best aptamer candidate and by its amplification. The process is summarized in Figure 1.

Library generation

The whole process for nucleic acid aptamer generation starts with the production of a synthetic library of a pool of ~10^{12}-10^{15} different oligonucleotides (ssDNA or RNA sequences), which are able to bind any target molecule. Each single sequence represents a possible aptamer candidate and is made of a central random region of ~25-30bp, flanked by two defined primers at the 3' and at the 5' ends, necessary for subsequent amplification (Ku et al, 2015; Sun and Zu, 2015). Aptamer generating libraries can be divided into five types, on the base of the collected sequences. The most common libraries are standard libraries which collect random sequences of 20-60 bp. Structurally-constrained libraries contain sequences with stable regions in order to induce aptamer folding in a certain secondary structure. Libraries based on a known sequence are constituted by oligonucleotides with specific and known sequences inserted in their random region. Finally, libraries based on genomic sequences (genomic SELEX) are created by digesting genomic DNA in order to find proteins capable of binding it (Vianini et al, 2001).

Binding and separation

After its generation, the library is incubated with aptamer target. A part of the oligonucleotides in the pool is able to recognize the target and these sequences are considered possible aptamers (partitioning), while unbound sequences are filtered out from the solution and discarded (elution) (Ku et al, 2015). Different methods have been developed in order to discriminate aptamers from specific sequences. First approaches, developed by Gold and co-workers, were based on a nitrocellulose membrane where the target was immobilized (Tuerk and Gold, 1990). However, alternative strategies based on biochemistry techniques have been developed to replace this approach, e.g., chromatographic affinity (Vianini et al, 2001; Levesque et al, 2007; Song et al, 2011) or magnetic columns (Niazi et al, 2008; Wang et al, 2008; Joeng et al, 2009) are often used. In addition, capillary electrophoresis has been proposed as a selection technique because of speed and high resolution, as has been demonstrated by Bowser and co-workers to select aptamer against neuropeptide Y and human IgE in only 4 rounds (Mendonsa and Bowser, 2004; Mendonsa

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and Bowser, 2005). Moreover, in recent years, aptamers have been selected against whole cells through the Cell-SELEX method, a complex technique, which allowed Gold and colleagues to select U251 glioblastoma cells (Daniels et al, 2003), and subsequently Kobatakes and colleagues to the identification of SBC3 lung cancer cells (Van Simaeys et al, 2010).

Amplification

After the selection of aptamers from a specific oligonucleotide pool, aptamers are amplified by polymerase chain reaction and the products of the amplification are used as new sub-library for the following selection rounds (Sun and Zu, 2015).

Downstream screening

After the first step of the SELEX, candidate aptamers are normally ~80 bp long. However, aptamer binding region is only 10-15 bp long (Gold et al, 1995; Jayasena et al, 1999) and the redundant nucleotides are deleted through a process called “aptamer truncation”. Many strategies have been developed in order to minimize aptamer length without affecting their binding regions, and most of them are based on computational biology. For example, Giangrande and colleagues truncated an RNA aptamer against the prostate-specific membrane antigen (PSMA) preserving its binding activity and its functionality through the use of a structure simulation and a target docking algorithms, while Green et al (1996) were able to select the shortest binding sequence of a DNA aptamer anti-platelet derived growth factor (PDGF) through partial fragmentation. Furthermore, other techniques have also been developed eluding the use of computational biology, e.g., Duan and co-workers selected the binding region of the anti-CD133 aptamer to recognize cancer stem cells through the hybridization with complementary oligonucleotides probes of non-essential regions (Zhou et al, 2011) as well as Wang and co-workers detected the anti-human protein tyrosine kinase 7 (hPTK7) (Shigdar et al, 2013). Such selection methods involving aptamer truncation are effective, however their complexity, the length and the cost remain a concern (Ku et al, 2015).

Aptamers in the biomedical field

The similarities between aptamers and monoclonal antibodies have prompted their use as potential therapeutics, and therefore several laboratories in academic and commercial settings are involved in developing aptamer-based treatments. The use of aptamers as potential drugs began with the approval of Pegaptanib by the USA Food and Drug Administration (US FDA) in 2004. Pegaptanib is a 27bp long RNA aptamer antagonist of the VEGF165 and is commercially available as Macugen® (Pfizer and Eyetech) (Eyetech Study, 2002; Eyetech Study, 2003; Lee et al, 2008), and is used for the treatment of the AMD, a degenerative disease that causes vision loss in older adults due to retinal damage. However, Macugen® seems to be effective and important also for the treatment of diabetic macular oedema (DME), proliferative diabetic retinopathy (PDR), ischemic diabetic macular oedema (MIDME), uveitis, choroid neovascularization secondary to pathologic myopia and iris neovascularization (NIH, 2008a; NIH, 2008b; Inc, 2010; Sultan et al, 2011; NIH, 2006).

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One of the most investigated aptamers is the TBA against thrombin, which was the first aptamer selected in 1992, and its efficiency was shown *in vivo* in 2012. After the demonstration of TBA efficiency *in vivo*, the Nu172 aptamers was developed by ARCA Biopharma as a potential thrombin inhibitor candidate and it is currently in phase II clinical trial to be certified as molecules suitable for anticoagulation during invasive medical procedures, such as coronary artery bypass graft and percutaneous intervention (Jo et al, 2006; Di Cera, 2007).

Aptamers also have application in oncology. Neoplastic progression could be often blocked through the inhibition of specific targets, which, if over-expressed, induces abnormal cell proliferation. Several clinical trials have indicated the usefulness of aptamers in binding tumour cells and inhibiting cancer development and progression. For example, Nucleolin, a protein often over-expressed on cancer cells surface and involved in cell survival, growth and proliferation, is a widely studied aptamer target (Bates et al, 2009). A 26 nucleotide long nucleolin-targeted DNA aptamer AS1411, developed and Antisoma Research and their academic colleagues, is in phase II clinical trials (Rosenberg et al, 2014) and has shown to be effective in inhibiting tumour (Bates et al, 1999; Ireson and Kelland, 2006).

APTAMERS-ENRICHED BIOMATERIALS

A wide range of strategies have been developed to enhance the bioactivity of materials. A common and promising method used both for DDS and for TE scaffolds, involves the immobilization of monoclonal antibodies on material surface. However, the use of monoclonal antibodies faces numerous issues, included immunogenicity, low stability and issues connected to their production (Jayasena, 1999; Eyetech Study, 2002; Eyetech Study, 2003; Ireson and Kelland, 2006; Xiang et al, 2015). As a consequence, alternative, such as aptamers, have grabbed attention as attractive alternatives to ameliorate the selectivity and thus the bioactivity of DDS and of TE scaffold for RM applications.

Bioactivity of aptamer-enhanced drug delivery systems

The ability of aptamers to bind their target with high affinity, together with the presence of over-expressed molecules on cancer cells surface, open the possibility to exploit aptamers as enhancer of DDS to improve chemotherapeutics selectivity and thus increase the possibility to achieve an active targeting (see Figure 2). Farokhzad et al (2004) pioneered this field; they synthesized poly(lactic acid)-block-polyethylene glycol NPs and bioconjugated an RNA aptamer anti-PSMA on NPs surfaces. Their results showed that Apt-NPs efficiently bind LNCaP cells better than NPs alone. On the other hand, when NPs were incubated with human PC3 prostate epithelial cells which do not express PSMA antigen low binding efficiency was found (Farokhzad et al, 2004). The same construct was used two years later to target Docetaxel in LNCaP xenograft tumour in nude mice. Results showed that Apt-DTXL-NPs were significantly able to reduce tumour volume if compared to DTXL-NP, Docetaxel alone, NPs alone or saline solution (Farokhzad et al, 2006). Later, Cao et al (2009) studied the *in vitro* effectiveness of liposomes conjugated with AS1411 aptamer and loaded with cisplatin against nucleolin over-expressing human breast cancer MCF-7 cells and against human prostate cancer LNCaP cells. They showed that after 4 days of culture, the viability of MCF-7 cells treated with Apt-LP-CP was significantly lower than that of MCF-7 cells treated with

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scramble aptamers liposome cisplatin-loaded system and of LNAcP cells treated with Apt-LP-CP (Cao et al, 2009). In this study, Cao and colleagues demonstrated that it is possible to activate target liposomes to specific cells and to inhibit their growth through the use of aptamers. Subsequently, Chen and co-workers (Guo et al, 2011) developed NPs derived from PLGA loaded with Paclitaxel and enriched with AS1411 aptamer. The anti-proliferation activity of the system was assayed *in vitro* on glioma brain C6 cells from rat. Apt-PTX-NP showed higher cytotoxicity and inhibition of cell growth if compared to NPs loaded with Paclitaxel or to Paclitaxel alone. After 24 hours the IC_{50} of Apt-PTX-NP was significantly lower than that of Apt-PTX and Taxol®, as well as after 48 hours (Apt-PTX-NP < PTX-NP < Taxol®) and after 96 hours (Apt-PTX-NP < PTX-NP < Taxol®) but with less significance. The anti-tumor efficacy of Apt-PTX-NP was subsequently evaluated *in vivo* in mice bearing glioma xenograft. After 8 days from the graft tumour volume began to decrease in mice treated with Apt-PTX-NP system. Moreover, the animals treated with Apt-PTX-NP system survived more than that of the PTX-NP, Taxol® saline control group (Guo et al, 2011). Moreover, In-Hyun et al described a DDS based on a dendrimer conjugated with an anti-PSMA aptamer and loads with Docetaxel. *In vitro* uptake of Apt-DOX-DEN system was higher than that of scramble Apt-DOX-DEN both for LNCaP and for human 22RV1 prostate carcinoma cells. The system was evaluated *in vivo* too on a 22RV1 xenograft tumour model and it showed a mark reduction of the tumour if compared to the saline control group (Lee et al, 2011).

Aptamer-enhanced scaffold bioactivity

As previously mentioned, it is very important to develop new methods to enhance scaffold biocompatibility and thus bioactivity in TE in order to obtain highly-dynamic scaffolds capable of interacting with autologous cells, and to positively modulate protein adsorption (Motta, 2005). In this section we want to focus on the possibility of using aptamers to improve bioactivity of biomaterial scaffolds.

Hoffmann et al (2007) suggested that the fast adhesion of circulating endothelial precursor cells (EPCs) on aptamer-coated vascular grafts could be useful to promote endothelium healing and to prevent eventual hyperplasia. They combined aptamers screened through Cell-SELEX against EPCs with vascular prostheses and tested their hypothesis by incubating aptamer-enriched implants with whole anti-coagulated porcine blood. After immuno-staining for CD31 and CD144[20], they observed that EPCs were captured on the enriched implants but not on controls without aptamers (Hoffmann et al, 2007). Five years later, Chen et al (2012) proposed another approach involving immobilised aptamers against surface cell receptors on PEG hydrogels to improve the ability of cells to adhere and to colonize the scaffold. They were able to show that cell proliferation was proportional to the concentration of aptamers used for functionalization (Chen et al, 2012). Similarly, we proposed the possibility of improving the biocompatibility of natural polymeric scaffolds by using ssDNA aptamers against fibronectin (Galli et al, 2016). Fibronectin is one of the major physiologically occurring proteins in damaged tissues and is mainly involved in cell adhesion and in regeneration process (Nuttelman et al, 2011). By using anti-fibronectin aptamers we aimed to ameliorate its adsorption on scaffolds and as a consequence to exploit it as a docking point for cell adhesion. The correct adsorption of fibronectin should allow a faster colonization of the scaffold *in vitro* and an accelerated regeneration *in vivo*.

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In Figure 3, we report the rationale behind enrichment of biomaterial scaffolds with aptamers against fibronectin. We chose to use two natural polymeric scaffolds as substrate: a thiolate hyaluronic acid/ di-acrylate polyethylene glycol hydrogel (tHA/PEGDA) and a chitosan modified with D-(+)-Raffinose film. Concerning the first material, tHA/PEGDA is a commercially available hydrogel distributed from the Sigma-Aldrich (Sigma-Aldrich, St.Louis, MI, USA) and normally used for stem cell culture, because it offers scant adhesion sites for cells. In our work we bound ssDNA aptamers to this hydrogel by exploiting the acrylate functional groups of PEGDA, which can easily bind the thiol groups on aptamers 3'-end. We were able to demonstrate that tHA/PEGDA hydrogels enriched with aptamers were able to bind selectively more fibronectin than controls and that the proliferation of human hOB osteoblast cells depended on the amount of aptamers used for the functionalization (Figure 4) (Galli et al, 2016). As for chitosan films, we chose to use chitosan because this polymer is highly investigated for TE applications, thank to its highly biocompatibility, biodegradability, low toxicity and ability to be moulded in a variety of shapes (Alves and Mano, 2008; Gasparini et al, 2014; Younes and Rinaudo, 2015). In this case, aptamers were immobilized on chitosan films by exploiting the ability of chitosan to spontaneously bind sulphur-containing compounds (Elviri et al, 2015). As for tHA/PEGDA hydrogels, chitosan enriched with aptamers supported cell proliferation of murine MC3T3-E1 osteoblast cells in a dose dependent manner (Figure 4).

Although the results we obtained are consistent, the rationale for their use can be different. Indeed, the results of the adsorption assay for fibronectin demonstrated that after the incubation of the scaffolds with or without aptamers with 30µg of serum proteins, the amount of fibronectin adsorbed on the substrates was different. The presence of aptamers on tHA/PEGDA hydrogels quantitatively increased the amount of adsorbed fibronectin and this may explain the amelioration in cell adhesion and proliferation. On the other hand, chitosan is known to bind massive amounts of protein from the supernatant and aptamers in fact do not alter the quantity of adsorbed proteins. We consequently think that aptamers on chitosan may affect the quality of adsorbed proteins. Aptamers may preserve the natural conformation of fibronectin on films during its adsorption, without unfolding it and maintaining a favorable exposure of adhesion sequences for cells.

CONCLUSIONS

Biomaterials have significant application in the field of biomedicine. They are used mainly as support for DDS and as substrate for TE scaffolds to improve their biocompatibility, and thus of their bioactivity. Several approaches proposed in the literature exploit the use of antibodies for specific target/molecular recognition to improve material bioactivity. However, the drawbacks associated with the use of antibodies have led to the development of alternative approaches such as nucleic acid aptamers. The exponential increase in research in the field of aptamers in the recent years and their many successful applications, it is clear that they can rival antibodies as molecules with specific binding characteristics.

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COMPETING INTERESTS

None declared.

LIST OF ABBREVIATIONS

3D: Three-dimensional
ADM: Wet Related **M**acular **D**egeneration
DDS: **D**rug **D**elivery **S**ystem
DME: **D**iabetic **M**acular **E**dema
ECM: **E**xtra**C**ellular **M**atrix
EPR: **P**ermeability and **R**etention **E**ffect
FDA: Food and **D**rug **A**dministration
hPTK7: **h**uman **P**rotein **T**yrosine **K**inase **7**
MIDME: **I**schemic **D**iabetic **M**acular **E**dema
PDGF: **P**latelet **D**erived **G**rowth **F**actor
PDR: **P**roliferative **R**etinopathy
PSMA: **P**rostate-**S**pecific **M**embrane **A**ntigen
RM: **R**egenerative **M**edicine
SELEX: **S**ystematic **E**volution **L**igands by **EX**ponential enrichment
TE: **T**issue **E**ngineering
VEGF: Vascular **E**ndothelial **G**rowth **F**actor

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FIGURES

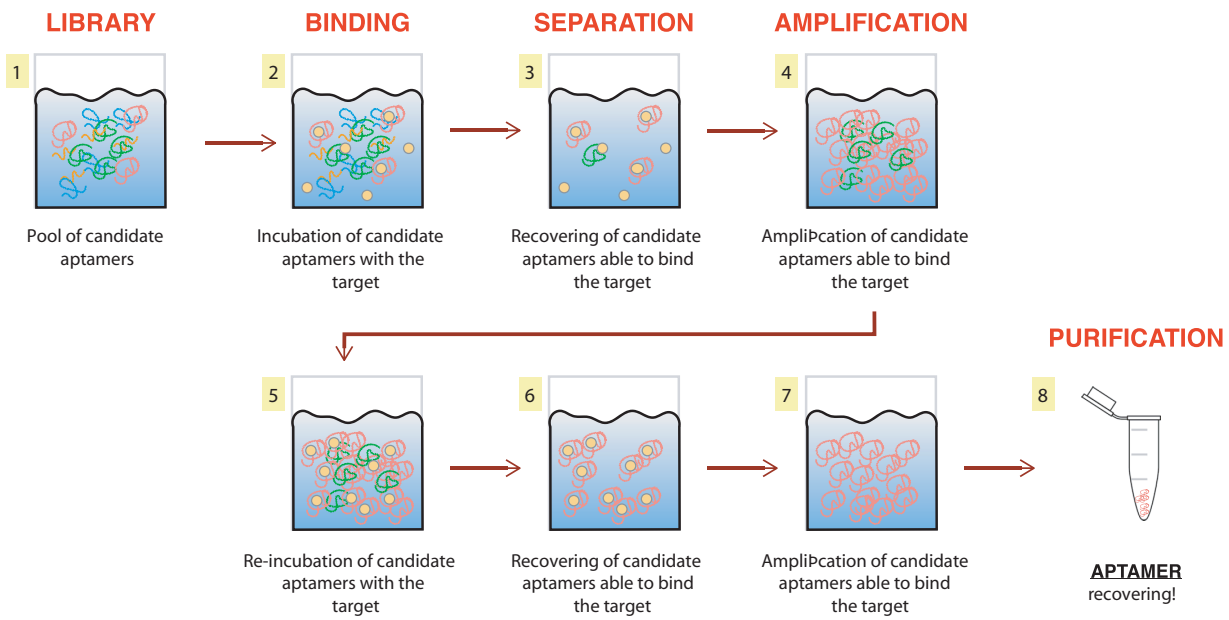

Figure 1. Flow diagram showing the process for aptamer generation.

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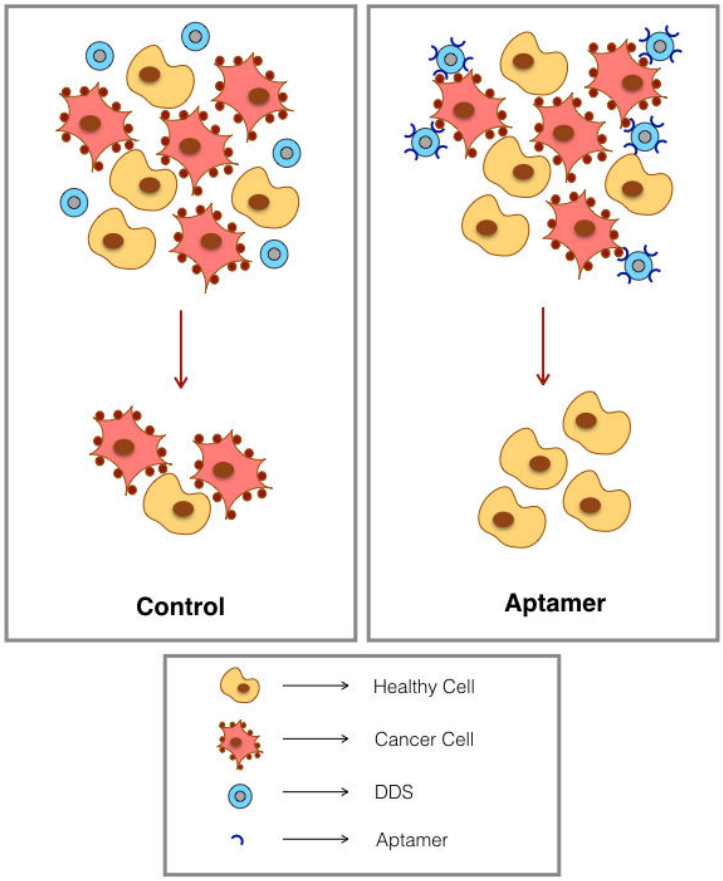

Figure 2. The principle of aptamer-decorated DDS. Control DDS release the drug, and kill cancer and neighboring cells around the tumor. Aptamer-enriched DDS specifically bind cancer cells and kill them selectively.

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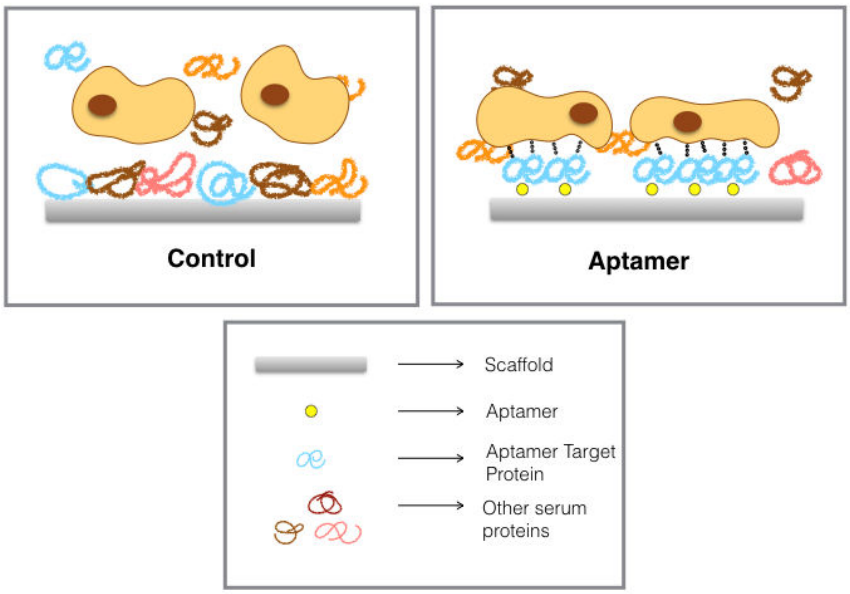

Figure 3. The use of aptamer-enriched scaffolds to retain specific proteins. Control scaffolds adsorb proteins from the environment based on their availability. Aptamer-enriched scaffolds specifically bind and retain target proteins, by selectively enriching their adsorption.

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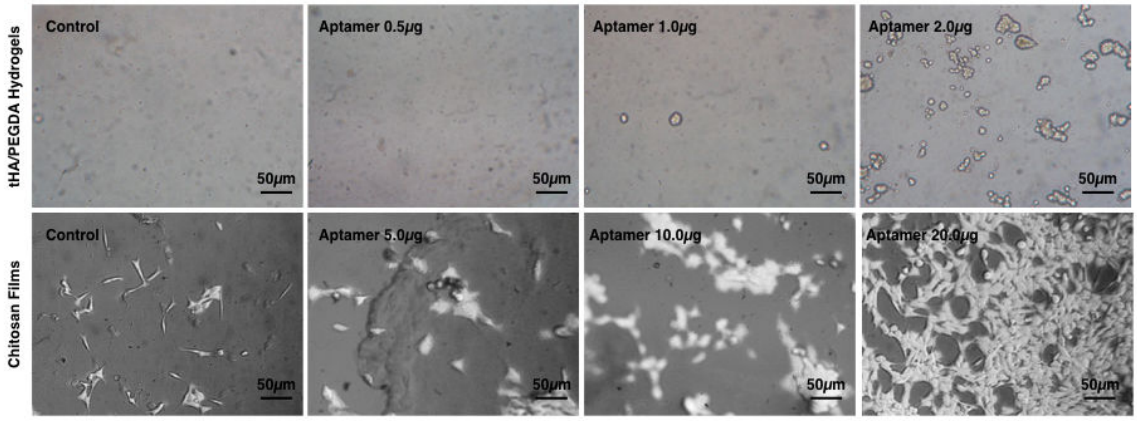

Figure 4. The ability of aptamers to improve cell proliferation and colonization of biomaterial scaffolds. The panels show human osteoblastic cells (hOb) and murine osteoblastic cells (MC3T3-E1) on tHA/PEGDA hydrogels and on 2% chitosan films, respectively, 24 hours after culturing at increasing doses of the aptamer.

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Tables

Table 1. Commercially available liposome formulations for chemotherapy (van Elk et al, 2016).

Product	Drug	Tumor
Doxil®/Caelyx®	Doxorubicin	Kaposi's Sarcoma Ovarian Cancer Breast Cancer Multiple Myeloma
Myocet®	Doxorubicin	Breast Cancer
DaunoXome®	Daunorubicin	Kaposis's Sarcoma
Marqibo®	Vincristine	Acute Lymphoblastic Leukemia

Table 2. Clinical Phase micelle formulations for chemotherapy (van Elk et al, 2016).

Formulation	Drug	Polymer	Tumor	Clical Phase
NC-6300	Epirubicin	PEG-b-poly(aspartate)	Solid Tumors	I
NK911	Doxorubicin	PEG-b-poly(a,B-aspartic acid)	Solid Tumors	II
NK105	Paclitaxel	PEG-b-poly(a,B-aspartic acid)	Gastric Cancer Breast Cancer	III
NC-4016	Oxaliplatin	PEG-b-poly(L-glutamic acid)	Solid Tumors	I
NK012	SN-38	PEG-b-poly(L-glutamic acid)	Breast Cancer	II
NC-6004	Cisplatin	PEG-b-poly(L-glutamic acid)	Pancreatic Cancer	III
BIND-014	Docetaxel	PEG-b-PLGA	Various	II
SP1049C	Doxorubicin	Pluronic L61 Pluronic F127	Various	II
Genexol-PM	Paclitaxel	mPEG-b-PDLLA	Various	IV