(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 August 2014 (28.08.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/128289 A1

(51) International Patent Classification:
A61L 27/34 (2006.01) *C12N 15/115* (2010.01)
A61K 31/7088 (2006.01) *C12Q 1/68* (2006.01)

(21) International Application Number:
PCT/EP2014/053502

(22) International Filing Date:
24 February 2014 (24.02.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
UD2013A000024 22 February 2013 (22.02.2013) IT

(71) Applicants: **LUMETTI, Simone** [IT/IT]; Via 27 Gennaio, 6, I-43125 Parma (IT). **MANFREDI, Edoardo** [IT/IT]; B.go della Posta, 11, I-43121 Parma (IT).

(72) Inventors; and
(71) Applicants : **GALLI, Carlo** [IT/IT]; Loc. Veggiola, 9, I-29025 Gropparello (IT). **MACALUSO, Guido Maria** [IT/IT]; Borgo Reale, 5, I-43121 Parma (IT).

(74) Agents: **PETRAZ, Gilberto Luigi** et al.; GLP SRL, Viale Europa Unita, 171, I-33100 Udine (IT).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(54) Title: BIOMEDICAL DEVICE IMPLANTABLE IN BONE AND/OR CARTILAGINOUS TISSUE, AND CORRESPONDING METHOD TO MANUFACTURE SAID BIOMEDICAL DEVICE

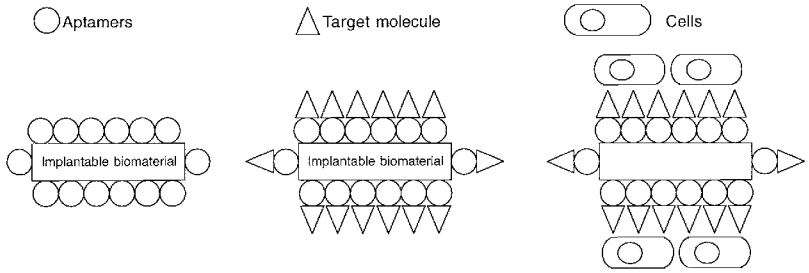

fig. 2

(57) Abstract: Aptamers for use in coating biomaterials, in particular for example for making biomedical devices which can be implanted in tissue, or tissue implants, corresponding method for coating biomaterials using aptamers and method for making biomedical devices which can be implanted in tissue, or tissue implants.

WO 2014/128289 A1

"BIOMEDICAL DEVICE IMPLANTABLE IN BONE AND/OR CARTILAGINOUS TISSUE, AND CORRESPONDING METHOD TO MANUFACTURE SAID BIOMEDICAL DEVICE"

* * * * *

FIELD OF THE INVENTION

Embodiments of the present disclosure relate to aptamers for use in coating biomaterials, in particular for example for manufacturing biomedical devices which can be implanted in bone and/or cartilaginous tissue, or tissue implants, a method for coating biomaterials using aptamers and a method for manufacturing biomedical devices which can be implanted in tissue, or tissue implants.

BACKGROUND OF THE INVENTION

Tissue regeneration

It is known that the regeneration of tissue defects caused by atrophy, trauma, pathological processes or as the result of previous surgery generally passes through the application, insertion or implantation of biomedical devices, which may or may not be reabsorbable, into the tissue.

Such devices have the characteristics of "scaffolds", that is, three-dimensional structures that, in contact with the surrounding tissue and with or without the presence of a surgical wound, promote the creation of a bond with said tissue, the colonization by cells of the nearby tissues, the marrow, the blood stream and the subsequent deposition of a new matrix and new mature tissue continuous with the already existing tissue and having the same histological and functional characteristics as the tissue present and the tissue lost.

Alternatively, or in addition to these procedures, implantable biomedical devices can be inserted into the tissue, whether healthy or damaged, to replace or complement a portion of organ or lost apparatus, as in the case of, but not limited to, a dental element, a joint, a vascular segment.

Fig. 1 shows the active principle of a reabsorbable scaffold for the regeneration of a tissue defect, in this case for example of the bone, according to passages 1 – 4. The defect (1) is surgically bridged with a material (2) that functions as a scaffold for the subsequent cell colonization (3) and the consequent formation of new tissue to replace the biomaterial, which is progressively reabsorbed.

The materials used for regenerative therapy can have an autologous origin, and

therefore may come from the same individual who is also the recipient; they may be homologous in nature and therefore come from an individual of the same species, or they may be heterologous and therefore originating from a living being of a different nature or synthetic type, and hence not deriving from a living being. Although autologous tissues can be re-implanted immediately into the recipient, and more correctly are called grafts, homologous materials and especially heterologous materials must first be processed in order to reduce the immune incompatibility with the receiving organism, possibly to eliminate the unwanted cell structures or components that could cause adverse responses. In the context of human bone, for example, bone tissues from cadaver donors can be frozen, freeze-dried, irradiated or even calcined. This last procedure eliminates the organic component using a high-temperature process, leaving only the mineral part, which is perfectly compatible. Examples of natural bone derivatives of a heterologous origin can be coral hydroxyapatites or natural apatites from calcined bovine, horse or pig bone. Synthesized materials like polymers, with a different nature and reabsorbability, have been tested and marketed, to prevent the problems of availability of original tissue and immune compatibility.

The success of a regenerative or rehabilitation therapy that provides to insert scaffolds or implantable devices to support prostheses, or prostheses themselves, is judged by whether a morphological and histological continuity of the tissue is restored through the deposition of newly-formed tissue in contact with the scaffold and possibly, if the latter is reabsorbable, its progressive replacement. To obtain this result, the material must be biocompatible, and hence it must be tolerated and must not cause adverse inflammatory and immune responses; it must not be toxic, and hence must not have components with toxic properties for the tissues of the organism and its conductivity, that is to say, it must be able to allow cell colonization, which allows the tissue to synthesize.

Biomimesis

A recent reformulation of this concept is summarized in the comprehensive term of biomimesis, that is, the capacity of a biomaterial to be integrated into the chemical and antigenic profile of the tissue into which it is inserted, offering the same type of signals and stimuli as the surrounding extra-cellular matrix. To create a biomimetic material, two types of approach have commonly been followed: the first provides to create favorable chemical-physical surface characteristics, and the second provides to

immobilize bioactive stimuli. In the first approach, the biomaterial is engineered so as to confer upon it chemical-physical characteristics that promote the spontaneous adsorption of proteins from the blood stream when the device is inserted into the wound. In fact, plasma contains a vast range of proteins, with the function of maintaining the osmotic properties of the blood, such as albumin, molecules useful for activating the coagulation cascade such as fibrinogen, adhesion molecules such as fibronectin or vitronectin, signal cytokines and chemokines such as inflammation mediators, hormones, nutrition molecules such as glucose or salts like calcium and phosphate. Such molecules can come into contact with any exogenous material introduced into the organism and tend to be adsorbed spontaneously thereon, by the formation of weak bonds, such as for example electrostatic interactions, dipole-dipole interactions or, more rarely, more stable bonds, depending on the nature of the biomaterial. The physical characteristics of the material, like hydrophilicity or wettability, influence for example the contact with the plasma, which is water-based, and the interaction with complex molecules like proteins which can comprise hydrophilic and hydrophobic residues. For this reason, following the spontaneous adsorption of the proteins, two events may occur. The first is that the concentration of the species adsorbed is proportional to the plasma concentration of the same. Therefore, most of the proteins adsorbed on a material, at least initially, consist of albumin, whereas the rare molecules such as cytokines, growth factors or hormones, are present to a lesser degree. Subsequently, however, since the adsorption is mediated by weak bonding forces, and is hence a generally reversible phenomenon, a balance is created between adsorption and detachment, and the concentration of adsorbed proteins with higher binding affinity gradually increases over time, based on the characteristics of the material implanted, in a way that is difficult to foresee and to control. The second phenomenon is fouling, or denaturation of the protein species adsorbed. That is, the proteins may be subjected during adsorption to a spontaneous alteration of their tertiary and/or quaternary structure, with the exposure of hidden domains and loss of functional activities of the protein or creation or exposure of epitopes with a new antigenic value and hence the possibility of activating immune phenomena of an innate or acquired nature. This can lead to adverse and even serious reactions, following the implantation of the device.

A second, although more complex, alternative approach, is to selectively enrich the surfaces of implantable biomaterials with proteins or molecules with a known bioactive

value, and favorable to the integration or functionality of the implant, such as for example growth factors. There is a great deal of available literature concerning enrichment of medical devices, for example of endosseous titanium implants by growth factors, such as Bone Morphogenetic Protein, which has a known capacity to induce cell differentiation in bone. In this way, following the insertion of the implant into bone, independently and irrespective of the spontaneous adsorption of plasma proteins on the body of the biomaterial, this introduces bioactive molecules to the colonizing cells which, coming into contact with the growth factor, are induced to differentiate according to the signal received. Structural molecules of the extra-cellular matrix, such as collagen, have also been often proposed for coating implantable surfaces because they offer numerous adhesion domains to cell attachment structures. This strategy, however, has some weaknesses. First of all the proteins must be extracted from tissue, which is a process that does not lack problems of an economic nature and availability of the original biological material; or they must be obtained through the application of recombinant DNA technology, generally through yeasts or other genetically modified microorganisms. In this case too, production is onerous and difficult. Moreover, some big proteins can cause adverse immune or inflammatory reactions and require a process of denaturation, which partly masks the antigenic nature thereof.

Document US-A-2009/148493 discloses devices with enhanced biocompatibility utilizing oligonucleotide functionalization, where biocompatibility is defined as the ability of an implanted device not to generate adverse responses in the receiving organism. Such document is mainly focused on the enhancement of cellular adhesion to the biomaterial.

Document US-A-2011/150964 discloses aptamer-coated implants for use as vascular implants, such as a stent or a pacemaker. The purpose of coating with aptamers in such document is to bind a specific subtype of cellular integrins, i.e. to directly bind molecules that mediate the cellular adhesion.

Publication "Immobilized DNA aptamers used as potent attractors for porcine endothelial precursor cells", Jan Hoffmann et al., Journal of Biomedical Materials Research, Part A, vol. 84A, no. 3, 16 July 2007, discloses aptamers developed against endothelial cells, to make devices that capture the endothelial cells circulating in blood, for example in order to isolate them.

There is therefore a need to improve biomedical devices, in particular for

implantation in bone or cartilaginous tissue to restore the anatomy or functionality of the bone or cartilaginous tissue, which overcomes at least one of the drawbacks of the prior art.

The purpose of the present invention is to overcome at least one of the shortcomings of the state of the art and to eliminate some or all the defects present therein, in particular to improve the regeneration of tissue defects and to improve the integration of implantable biomedical devices by coating with biomaterials, used to refill the defect or to construct the implantable devices themselves.

The Applicant has devised, tested and embodied the present invention to overcome the shortcomings of the state of the art and to obtain these and other purposes and advantages.

Unless otherwise defined, all the technical and scientific terms used here and hereafter have the same meaning as commonly understood by a person with ordinary experience in the field of the art to which the present invention belongs. Even if methods and materials similar or equivalent to those described here can be used in practice and in the trials of the present invention, the methods and materials are described hereafter as an example. In the event of conflict, the present application shall prevail, including its definitions. The materials, methods and examples have a purely illustrative purpose and shall not be understood restrictively.

SUMMARY OF THE INVENTION

The present invention is set forth and characterized in the independent claims, while the dependent claims describe other characteristics of the invention or variants to the main inventive idea.

In accordance with the above purposes, forms of embodiment described herein provide aptamers for use in coating biomaterials, for manufacturing a biomedical device configured to be implanted in bone or cartilaginous tissue, to restore the anatomy or functionality of the bone or cartilaginous tissue. According to some aspects, the biomaterials in question are suitable to make a biomedical device implantable in bone or cartilaginous tissue.

According to aspects, a biomedical device is provided, which is configured to be implanted in bone or cartilaginous tissue to restore the anatomy or functionality of said bone or cartilaginous tissue. The biomedical device comprises one or more biomaterials coated with aptamers, wherein the biomaterial is chosen from a group comprising:

collagen, hydrogel, fresh or processed autologous tissue, processed homologous or heterologous tissue, a fresh or processed tissue extract, demineralized bone matrix, natural apatite, organic sponge, implantable matrix, bioceramics, bioglass, endosseous or orthopedic implant, an implantable prosthesis, a sensor, a biosensor, a metal, a metal alloy or any combination thereof.

In some forms of embodiment, the one or more aptamers can include polymers of D-deoxyribonucleic acid, D-ribonucleic acid, L-deoxyribonucleic acid, L-ribonucleic acid or oligopeptides.

In some forms of embodiment, the one or more aptamers are selected to bond target molecules and/or to bond nucleic acids, comprising for example oligonucleotides, Plasmid DNA, fragments of chromosome DNA, RNA, siRNA, shRNA and/or to bond exogenous molecules, including drugs.

Forms of embodiment described here also provide a compound comprising at least one biomaterial and one or more aptamers which at least coat at least one biomaterial, wherein optionally the one or more aptamers are present only on the surface of the biomaterial or in the whole thickness of the biomaterial.

In some forms of embodiment, the one or more aptamers are bonded, optionally by spacers or crosslinkers, to the biomaterial by the 5', 3' end, or both.

In some forms of embodiment, the one or more aptamers are bonded to the biomaterial by functional groups provided on the biomaterial.

In some forms of embodiment, the compound comprises a composite material formed by the combination of a plurality of biomaterials coated with the one or more aptamers.

In some forms of embodiment, the compound comprises at least two aptamers joined together, of which one aptamer is configured to recognize a recognition portion of the biomaterial, and another aptamer is configured to remain free so as to perform a biomimetic function.

In some forms of embodiment, the at least one biomaterial is functionalized with a single type of aptamer or with a mixture of two or more types of aptamers.

In some forms of embodiment, the compound also comprises additional molecules with a pharmacological function.

Forms of embodiment described here concern a method to manufacture biomedical devices implantable in bone or cartilaginous tissue, or tissue implants, to restore the

anatomy or functionality of the bone or cartilaginous tissue. The method provides to at least coat at least one biomaterial using one or more aptamers according to the present disclosure.

According to aspects, the method provides that the biomaterial is chosen from a group comprising: collagen, hydrogel, fresh or processed autologous tissue, processed homologous or heterologous tissue, a fresh or processed tissue extract, demineralized bone matrix, natural apatite, organic sponge, implantable matrix, bioceramics, bioglass, endosseous or orthopedic implant, an implantable prosthesis, a sensor, a biosensor, a metal, a metal alloy or any combination thereof.

In some forms of embodiment, the biomaterial is functionalized with said one or more aptamers against a target molecule.

In some forms of embodiment, the method provides to modify the one or more aptamers by introducing modifications to the nitrogenous bases, to the sugars or to the ligands that form the nucleotides, such as for example modifications to the cytidine 2', and uracil, or through capping of the ends.

In some forms of embodiment, the method provides to bond, directly or by spacers or crosslinkers, to one 5', 3' end or to both ends of the oligonucleotide chain of the one or more aptamers, at least one functional group chosen from: amino groups, sulfhydryl groups, carboxylic groups, azides, vinyl sulfones, acrylics, methacrylics, hydroxylics, phosphorics, maleimides, N-hydroxysuccinimides, benzoyls, 5-bromoUracil or 5-iodoUracil.

In other forms of embodiment, the method provides to keep free one end of the one or more aptamers not bonded to the biomaterial, or to subject to capping one end of the one or more aptamers, or to bond, directly or using spacers, an accessory molecule to one end of the one or more aptamers, wherein optionally the accessory molecule is a molecule configured to increase the efficacy of the aptamer, to confer accessory biological properties, to improve the resistance of the aptamer to the action of enzymatic degradation or to verify and control the quality of the material, wherein optionally said accessory molecule is chosen from a group comprising: biotin, a fluorophore, an oligopeptide, a polypeptide with enzyme activity and/or signal activity and/or adhesive activity.

In some forms of embodiment, the method provides to bond the one or more aptamers to the biomaterial by means of functional groups provided on said biomaterial

and chosen from a group comprising: amino groups, sulfhydryl groups, carboxylic groups, azides, vinyl sulfones, acrylates, hydroxylics, phosphorics, maleimides, N-hydroxysuccinimides, benzoyls.

In some forms of embodiment, the method provides to combine a plurality of biomaterials to obtain a composite material that is coated with one or more aptamers.

In some forms of embodiment, the method provides to join at least two aptamers, of which one aptamer recognizes a recognition portion of the biomaterial and another aptamer remains free so as to perform a biomimetic function.

In some forms of embodiment, the method provides to functionalize the at least one biomaterial with a single type of aptamer or with a mixture of two or more types of aptamers.

In some forms of embodiment, the method provides to add additional molecules with a pharmacological function to a compound formed by the at least one biomaterial coated with the one or more aptamers.

Still other forms of embodiment described here concern a method for coating biomaterials that provides to use aptamers to coat at least one biomaterial.

According to embodiments described herein, a method is provided for increasing osteogenicity and/or chondrogenicity of biomaterials implantable in bone and/or cartilaginous tissues by aptamers according to the present disclosure.

According to embodiments described herein, a method is provided for increasing formation of bone and/or cartilaginous tissues by aptamers according to the present disclosure.

According to embodiments described herein, a method is provided for increasing regeneration of bone and/or cartilaginous tissue by aptamers according to the present disclosure.

These and other features, aspects and advantages of the present disclosure will become better understood with reference to the following description, the drawings and appended claims. The drawings, which are incorporated in and constitute a part of this specification, illustrate embodiments of the present subject matter and, together with the description, serve to explain the principles of the disclosure.

DETAILED DESCRIPTION OF SOME FORMS OF EMBODIMENT

Forms of embodiment described here concern aptamers for use in coating biomaterials, for making biomedical devices which can be implanted in tissue, or tissue

implants, a method to make said biomedical devices implantable in tissue and a method for coating biomaterials using aptamers. According to some aspects, aptamers are provided for coating biomaterials suitable for tissue implantation, the latter also called hereafter "scaffolds" or "vector scaffolds".

In the present description, the term "tissue" shall be taken to mean a biological material that makes up the organs of the human or animal body, formed by an aggregation of cells that have similar shape, structure and functions and, generally, a common embryological origin, such as for example, but not restrictively, epithelium tissue, connective tissue, muscular tissue, nervous tissue, bone tissue, cartilaginous tissue, parenchymal tissue or others.

According to the present disclosure, biomedical device are provided which are configured to be implanted in bone or cartilaginous tissue to restore the anatomy or functionality of the bone or cartilaginous tissue, comprising one or more biomaterials coated with aptamers, wherein the biomaterial is chosen from a group comprising: collagen, hydrogel, fresh or processed autologous tissue, processed homologous or heterologous tissue, a fresh or processed tissue extract, demineralized bone matrix, natural apatite, organic sponge, implantable matrix, bioceramics, bioglass, endosseous or orthopedic implant, an implantable prosthesis, a sensor, a biosensor, a metal, a metal alloy or any combination thereof.

According to the present disclosure, possible biomedical devices which can be implanted in bone or cartilaginous tissue can be for instance:

- cartilaginous or bone substitutes, i.e. biomaterials which can be implanted in tissue, in case of lack of tissue, and which can substitute the lacking tissue, providing regeneration of the lacking tissue starting from the surrounding tissue, generally being replaced at the end of the regeneration by the same tissue;
- implants, such as orthopedic or dental implants, for integrating the functionality of a missing element, such as bone portion, e.g. hip prosthesis, or lacking teeth, e.g. dental implants.

Basically, the inventive idea underlying the present disclosure is to coat biomaterials with aptamers (DNA or RNA oligonucleotides and their respective levo-isomers) designed for different target molecules, so as to sequester them on or in the biomaterial, by eliciting specific function on the cells colonizing such biomaterials or implantable medical device.

According to embodiments, which can be combined with all embodiments described herein, bone substitutes can preferably comprise porous biomaterials.

According to possible implementations, porous biomaterials are preferably permeable to nutrients and growth factors required for bone growth. During wound healing oxygen and nutrients supply depends exclusively on blood vessels in the tissues surrounding the defect until new vessels have been formed in the newly formed tissue. Therefore the survival of cells in the center of the defect is limited by how easily oxygen and nutrients can permeate the defect and the biomaterials that have been placed in it. A porous structure makes it easier for oxygen and nutrients to diffuse and are also more easily resorbed because a greater surface is exposed to bone cells, such as osteoblasts and osteoclasts. Porous scaffolding materials, according to embodiments described herein, can comprise pores having diameters ranging from about 1 μm to about 1 mm. In embodiments, a porous material according to the present disclosure comprises macropores having diameters ranging from about 100 μm to about 1 mm. In further embodiments, a scaffolding material comprises mesopores having diameters ranging from about 10 μm to about 100 μm. In still further embodiments, a scaffolding material according to the present disclosure comprises micropores having diameters less than about 10 μm. Embodiments described herein contemplate scaffolding materials comprising macropores, mesopores, and micropores or any combination thereof. According to embodiments described herein, the porous biomaterial can have any shape and dimension, according to bone substitutes and implantable devices commonly used in the dental or orthopedic practice. It can be in the form of a single device or blocks or granules, or a powder, irrespective of the granule size, with a minimum of at least 1 micron.

Fig. 2 is used to describe forms of embodiment of a design of the mechanism on which the present description is based, wherein for example the implantable biomaterial is functionalized with aptamers against a target molecule that can facilitate cell adhesion and/or growth and/or functionality.

In some forms of embodiment, which can be combined with all the other forms of embodiment described here, the biomaterial can be chosen from a group comprising: collagen, hydrogel, fresh or processed autologous tissue, processed homologous or heterologous tissue, a fresh or processed tissue extract, demineralized bone matrix, natural apatite, organic sponge, implantable matrix, bioceramics, bioglass, endosseous

or orthopedic implant, an implantable prosthesis, a sensor, a biosensor, a metal, a metal alloy or any combination thereof.

According to embodiments, which can be combined with all embodiments described herein, endosseous implants can be selected in a group consisting of: implantable device for dental or orthopedic use, such as a titanium or zirconia endosseous implant, herein simply referred to as "endosseous implant". Titanium and zirconia implants are common devices used to support different kind of prosthetic devices, such as dental prosthesis, which can used according to the present disclosure. They are generally constituted by a cylindrical or conical ("root-form") threaded device, which can be inserted and retained in bone tissue after healing. In embodiments described herein implant can be processed by CAD/CAM techniques, according to commonly used techniques in the art, to obtain the desired shape to match a tissue defect or to perform a certain therapy.

According to embodiments, which can be combined with all embodiments described herein, bioceramics can be selected in a group consisting of: monocalcium phosphate monohydrate (MCPM), dicalcium phosphate (DCP), tricalcium phosphate (TCP), amorphous calcium phosphate (ACP), hydroxyapatite (HA), tetracalcium phosphate (tetCP), fluorapatite, calcium carbonate, calcium sulphate or a combination thereof.

In some forms of embodiment, the biomaterial can comprise or consist of calcium-phosphate bioceramics. These materials can be inorganic biomaterials with a crystalline structure formed by calcium, phosphate and additional substitute ions, which mimic the composition of the bone tissue. Some examples of calcium-phosphate bioceramics usable as scaffolds according to the present invention are amorphous calcium phosphate, monocalcium phosphate monohydrate (MCPM), monocalcium phosphate anhydrous (MCPA), dicalcium phosphate dihydrate (DCPD), dicalcium phosphate anhydrous (DCPA), octacalcium phosphate (OCP), α–phosphate tricalcium, β-phosphate tricalcium, hydroxyapatite (HA), poorly crystalline hydroxyapatite, tetracalcium phosphate (TTCP), heptacalcium decaphosphate, calcium metaphosphate, calcium pyrophosphate dihydrate, calcium pyrophosphate, calcium phosphate carbonate, or a combination of these.

In possible examples, the biomaterial can therefore be natural or synthetic, totally or partly, it can have the form of a gel, such as, but not exclusively, a collagen gel or a polyethylene glycol gel, a liquid, sponge, or blocks, such as blocks of synthetic or

natural hydroxyapatite or a polymer of polylactic acid, or polyglycolic acid or a copolymer of the same, granules, a biomedical device with the predetermined shape depending on the function to be performed, such as a dental or orthopedic endosseous implant or a coronary stent, a prosthesis to replace an organ or a part of an organ, such as a valve, ophthalmic or vascular prosthesis.

In possible examples the material may comprise or consist of biodegradable synthetic polymers such as polyglycolic acid, polylactic acid, polycaprolactic acid, polytrimethylene carbonates, polyhydroxybutyrates, polyhydroxyvalerates, polydioxanones, polyorthoesters, polycarbonates, poly-tyrosine carbonate, poly ortho-carbonates, polyalkylene oxalates, polyalkylene succinats, polymalic acids, polymaleic anhydrides, polypeptides, polydepsipeptides, polyvinyl alcohol, polyesteramides, polyamides, polyanhydrides, polyurethanes, polyphosphazenes, polycyanoacrylates, polyfumarates, polyamino acids, modified polysaccharides (such as cellulose, starch, dextran, chitin, chitosan, etc.), modified proteins (such as collagen, casein, fibrin, etc.) and their copolymers, terpolymers or combinations or mixtures of polymers. Collagen may also be used for the construction of scaffolds because of its biocompatibility and favorable characteristics for supporting cell adhesion and functionality (see for example US Patent n. 5,019,087). Collagen sponges for clinical use are an example of scaffold and are well known (e.g. Gingistat).

In some forms of embodiment, the scaffold can comprise, consist of or essentially consist of a reabsorbable membrane. One example of a reabsorbable membrane available on the market is BioGide . One example of a reabsorbable membrane available on the market is ePTFE GoreTex .

The biomaterial can have a predefined shape when it is inserted or it can be polymerized before, during or after insertion into the receiving organism with mechanisms using chemical or thermal initiation, or by means of reactive photo-initiators on different wavelengths.

In possible implementations, polymeric biomaterials which can be used according to the present disclosure can be for instance: polyethylen glycole derivatives, polyvinyl alcohol derivatives, dextran derivatives, chitosan derivatives, hyaluronic acid derivatives, collagen derivatives, alginate derivatives, polyurethane derivatives, or a combination thereof. polylactic acid (PLA); polyglycolid acid (PGA); copolymers of lactic acid and glycolic acid (PLGA); polycaprolactone; polyphosphoester;

polyorthoester; poly(hydroxy butyrate); poly(diaxanone); poly(hydroxy valerate); poly(hydroxy butyrate-co-valerate); poly(glycolide-co-trimethylene carbonate); polyanhydrides; polyphosphoester; poly(ester-amide); polyphosphoeser; polyphosphazene; poly(phosphoester-urethane); poly(amino acids); polycyanoacrylates; biopolymeric molecules such as fibrin; fibrinogen; cellulose; starch; collagen; and hyaluronic acid; or a mixture or a copolymer thereof.

In some forms of embodiment, the scaffold may comprise, consist or essentially consist of photo-polymerizable materials with visible or ultraviolet (UV) light wavelengths.

Some types of matrices or hydrogel can be photo-polymerized in vivo and in vitro in the presence of photo-initiators using visible or UV (ultraviolet) light. Some non-restrictive examples of water-soluble photopolymerizable polymers or photo-polymerizable hydrogels are PEG acrylate derivatives, PEG methacrylate derivatives, polyvinyl alcohol (PVA) derivatives, modified polysaccharides such as of hyaluronic acid derivatives and methacrylate dextran derivatives, and a photo-initiator as described in literature (Nhuyen and West, Biomaterials 23; 2002). Photo-polymerizable materials can also be copolymers, that is, formed by a mixture of different monomers. Visible or UV light can interact with light-sensitive compounds called photo-initiators to create free radicals able to initiate the polymerization of the crosslinked hydrogel. Some non-restrictive examples of photo-initiators can include 1-[4-(2- hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one, acetophenone, benzophenone, and benzoin ethers. Other non-limiting examples are 2,2-dimethoxy-2-phenylacetophenone, benzoin methyl ether, as described by H. Singer for example in US Patent n. 4620954. One example of a commercially available UV photo-initiator is Irgacure® 2959®. Some non-restrictive examples of visible light photo-initiators are eosin Y, or triethanolamine. N-vinylpyrrolidinone (NVP) can be used as a monomer and accelerator for photo-polymerization/photocrosslinking. Other examples of accelerators include N1N dimethyl toluidine or tetramethylethylenediamine. Preferably, the photo-initiators and accelerators are cyto-compatible.

APTAMERS

Aptamers usable in forms of embodiment described here can typically be molecules consisting of short polymers of D-deoxyribonucleic acid, D-ribonucleic acid, their levo chiral isomer equivalent and oligopeptides. Aptamers consisting of L-nucleotides are

also known as "spiegelmers". These short chains of D- or L-nucleotides, generally single strand, in the range usually comprised between 30 and 70 nitrogenous bases, are able, by means of intra-molecular couplings between the complementary bases or their modified analogs (2'-Fluorine, 2'-O-Methyl, 2'-Amino, Phosphorothiates or others), to fold into a secondary structure with double helical portions (stem) connected by segments or single strand loops. This three-dimensional folding, obligated by the combination of various secondary foldings on the part of multiple portions of the molecule of the aptamer, allows them to bond, by means of a conformational matching, with a target protein. The single strand portions (loops) represent the most common recognition regions, through the formation of hydrogen links or through interactions between planar aromatic groups. Often the target-aptamer interactions are stabilized by the formation of compounds with metal ions, such as Mg^{2+} or Mn^{2+}.

The method for selecting the aptamer sequences desired for predefined purposes is through some selection protocols used commercially, among which the SELEX method (systematic evolution of ligands by exponent enrichment) is one of the most widespread for oligonucleotide screening. In short, this protocol provides to make the target protein, decided in advance, react with a library of nucleotide sequences of the desired species (DNA, RNA or other). Following conformational matching, some molecules will bond with the target, and will be separated from the non-bonded sequences and amplified by PCR. Successive cycles of the operation, on average from 5 to 10, allow to select some candidates which show greater bonding affinity with the target molecule. The instruments and procedures for screening are available commercially and known. Each procedure for selecting aptamers can be used to generate the desired aptamers, since the selection procedure does not influence the aptamers produced.

Generally speaking, aptamers can be used as substitutes for antibodies, which are peptides generated by the plasma cells, for the purposes of diagnosis and research, to detect for example the presence of a target molecule in a biological sample, or have been proposed for therapeutic purposes to sequester and remove from affected tissues proteins important for the development of the pathology, such as thrombin in conditions favoring thrombosis, or growth factors or products of oncogenes in certain types of tumors, or viral proteins in certain types of infection, following complexation of the aptamer, often if not constantly, with molecules such as PEGs. One example of aptamer (RNA) proposed for therapy is Pegaptanib (Macugen-OSI Pharmaceuticals), used for

treating macular degeneration (AMD) and diabetic macular edema (DME), or ARC1779, an aptamer that functions as a competitive antagonist of the VonWillebrand Factor (VWF) to be used in cases of acute coronary syndrome (ACS). Aptamers possess some advantages compared to antibodies. First of all their in vitro selection process is totally controllable compared with the use of laboratory animals needed to produce antibodies, irrespective therefore of the multiple mechanisms of an immune response that are activated in the generation of antibodies. Moreover, antibodies function only in physiological conditions, whereas aptamers, at least those based on DNA, possess a considerable stability at high temperatures and can easily be recovered after heat denaturation, they can be optimized and manipulated on a level of the target interaction region.

In some forms of embodiment, which can be combined with all the other forms of embodiment described here, the one or more aptamers usable can be chosen from a group comprising: single or double stranded D-deoxyribonucleic acid, single or double stranded D-ribonucleic acid, their levo chiral isomer equivalent or oligopeptides.

For example, the aptamers can be selected using known methods in common use in the field among aptamers that bond molecules, proteins or not, human or animal molecules of biological interest, intracytoplasmatic or extracytoplasmatic, present in plasma as fibrinogen or albumin or globulin, or in the tissue to be regenerated or in other tissues of the organism with structural function, such as for example, but not only, Collagen, Laminin, Elastin, Fibronectin, Vitronectin, F-spondin, Periostin, Thrombospondin, proteoglycans such as Heparan-sulfate, Chondroitin sulfate, Keratan sulfate, Hyaluronic acid, or as signal function, including cytokines such as IL-1, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-16, IL-17, IL-23, IL-34, IL-35, Tumor necrosis factor (TNF), Interferon, chemokine, such as but not only CCL1, CCL2, CCL3, CCL5, CCL6, CCL7, CCL8, CCL9, CCL11, CCL12, CCL13, CCL14, CCL1, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, Osteoprotegerin, RANKL, Sclerostin, DKK, sFRP, immunoglobulin, or growth factors, such as for example Adrenomedullin (AM), Angiopoietin (Ang), Autocrine motility factor, Bone morphogenetic proteins (BMPs, such as BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, or BMP7), Brain-derived neurotrophic factor (BDNF), Epidermal growth factor (EGF), Erythropoietin (EPO), Fibroblast growth factor (FGF), Glial cell line-derived neurotrophic factor (GDNF), Granulocyte colony-stimulating

factor (G-CSF), Granulocyte macrophage colony-stimulating factor (GM-CSF), Growth differentiation factor-9 (GDF9), Human growth/differentiation factor-5 (GDF-5), Hepatocyte growth factor (HGF), Hepatoma-derived growth factor (HDGF), Insulin-like growth factor (IGF), Migration-stimulating factor, Myostatin (GDF-8), Nerve growth factor (NGF), Platelet-derived growth factor (PDGF), Thrombopoietin (TPO), Transforming growth factor (TGF, such as TGF-β1 TGF-β2, TGF-β3, TGF-β4, TGF-β5 or any other member of the TGF-β family), Vascular endothelial growth factor (VEGF), Wnt family growth factors, Placental growth factor, (PlGF), or hormones, such as but not only parathyroid hormone (PTH) or fragments thereof, Growth Hormone (GH), Insulin, Calcitriol, Estrogens, Progesterone, Prolactin, Testosterone, or components of the signal cascades, proteins such as Amelogenin.

In some forms of embodiment, the aptamers can bond exclusively with an isoform of the target protein, or can be cross-reactive with different isoforms, or with homologous forms of the target of different species, or with fragments of the target, or with individual domains of the target, or with modified forms or fragments of the target.

In some forms of embodiment the aptamers bonded with the biomaterial can bond with nucleic acids, comprising but not limited to oligonucleotides, plasmid DNA, fragments of chromosome DNA, RNA, siRNA, shRNA, and can serve to convey the same for therapeutic purposes.

In some forms of embodiment the aptamers can bond with proteins that have in their turn a vector function for said nucleic acids, like Cell Penetrating Peptides, that can convey the nucleic acids in situ.

In some forms of embodiment, the aptamers can bond with exogen molecules, such as for example drugs with different functions such as, but not limited to, chemotherapy drugs, antibiotics, drugs that promote scar formation, the regeneration of tissue, the functionality of tissue, which can be loaded onto the scaffold-aptamer compound before insertion into the organism or subsequently administered to the receiving subject and captured by the aptamer thanks to local diffusion or through the blood stream.

According to embodiments, the aptamers can bond to molecules with signal function which are subsequently recognized and then stimulate cells colonizing the biomaterial and the implantable medical device, or, in further embodiments, the aptamers can directly bond with cell receptors.

In some forms of embodiment the aptamers can bond directly with membrane cell

receptors for cytokines, growth factors or hormones present on the cells of the tissue, or of other tissues, or exogen cells supplied for the purpose of therapy, and to contribute to the activation of signal ways therein and to the modulation of the cell activity.

In some forms of embodiment, the aptamers can be modified in their structure, typically to improve one or more of the following properties: efficacy, functionality, affinity of the ligand, tolerability, half-life or resistance to the action of enzymatic degradation.

In some forms of embodiment the aptamers can consist of oligonucleotides. An oligonucleotide can comprise natural nucleosides (adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine); analogs of nucleosides (for example 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyladenosine, 5-methylcytidine, C-5 propionyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propionyl-uridine, C5-proprionyl-cytidine, C5-methycytidine, 7-deazaadenosine, 7-deazaguanosine, 8-oxyadenosine, 8-oxyguanosine, O(6)-methylguanine, and 2-thiacytidine); chemically modified bases; biologically modified bases (for example methylate bases); intercalated bases; modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxiribose, arabinose, and hexose); and/or modified phosphate groups (for example phosphorothioates).

Chemical modifications of the nucleotides, if present, can include for example, individually or in any combination, those described in documents US Patent n. 7964365 or US Patent n. 0253243. These include but are not limited to: modifications of sugar in position 2', modifications of pyramidine in position 5' (for example 5-(N-benzylcarboxiamide)-2'-deoxyuridine, 5-(N-isobutylcarboxiamide)-2'-deoxyuridine, 5-(N-tryptaminocarboxyamide)-2'-deoxyuridine, 5-(N-[1-(3-trimethylammonium) propyl]carboxyamide)-chloro 2'-deoxyuridine, 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine, or 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine), modifications of exocyclic amines, substitution of 4 thyouridine, substitution of 5-bromo- or 5-iodouracil, backbone modifications, methylations, couplings of unusual bases as isobases, isocytodine and isoguanidine, and suchlike.

Other modifications can include modifications of elements among nucleotides for example, modifications that involve chargeless ligands like methylphosphonates, phosphototriesterases, phosphoramidites, carbamates, etc. and charged ones such as

phosphorothioates, phosphorodithioates etc., those with intercalating agents (for example acrydine, psoralen, etc.), those with chelants (for example metals, radioactive metals, boron, oxidizing metals, etc.), those containing alkilants and those with modified ligands (for example alpha anomeric nucleic acids, etc.).

Moreover, each of the hydroxyl groups normally present in a sugar can be: substituted by a phosphonate group or phosphate; protected by standard protector groups; or activated to form other bonds to other nucleotides or to a solid support. The terminal groups OH 5' and 3' can be phosphorylated or substituted by amines, with organic caps with a length from 1 to about 20 carbon atoms, or organic caps with a length from 1 to about 20 polymers of polyethylene glycol (PEG) or other biological polymers or hydrophilic or hydrophobic synthetic polymers. A modification to the nucleotide structure, if present, can be created before or after the assembly of the polymer. A sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can also be modified after polymerization, such as for example by conjugation with a marker.

Polynucleotides can also contain analogous forms of generally known ribose or deoxyribose, including 2'-O-methyl-, 2'-O-allylic, 2'-fluorine- or 2'-azido-ribose, analogous to carbocylic sugars, α-anomeric sugars, epimeric sugars such as arabinose, xylose or lyxose, pyranose sugars, furanose sugars, sedoheptuloses, analogous acyclics and analogous to abasic nucleotides like methyl riboside. One or more phosphotriesterase ligands can be replaced by alternative ligand groups. Alternative ligand groups include embodiments where phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithiolate"), (O)NR2 ("amidate"), P(O)R', P(O)OR', CO or CH2 ("formacetal"), where every R or R' is independently H or a substituted or non-substituted alkyl (from 1 to 20 C atoms) which can also contain an ether ligand (—O—) linkage, arylic, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all the ligands in a polynucleotide must necessarily be identical. The substitution of analogous forms of sugars, purines and pyrimidines can be advantageous in designing a final product, like alternative structures of the backbone or polyamide backbone, for example.

In some forms of embodiment, the oligonucleotide contains, consists of or essentially consists of photo-reactive nucleotides or photo-reactive pyrimidines. The term "photo-reactive nucleotides" refers to every modified nucleotide which is capable of photo-crosslinking with a target, like a protein, following irradiation with a source of light at a

determinate wavelength. For example, photo-aptamers produced by a photo-SELEX process can include a photo-reactive group like the following: 5-bromouracil (BrU), 5-iodouracil (IU), 5-bromovinyluracil, 5-iodovinyluracil, 5-azidouracil, 4-thiouracil 5-bromocytosine, 5-iodocytosine, 5-bromovinylcytosine, 5-iodovinylcytosine, 5-azidocytosine, 8-azidoadenine, 8-bromoadenosine, 8-iodoadenosine, 8-azidoguanine, 8-bromoguanine, 8-iodoguanine, 8-azidohypoxanthine, 8-bromohypoxanthine, 8-iodohypoxanthine, 8-azidoxanthine, 8-bromoxanthine, 8-iodoxanthine, 5-bromodeoxyuridine, 8-bromo-2′-deoxyadenosine, 5-iodo-2′-deoxyuracil, 5-iodo-2′-deoxycytosine, 5-[(4-azidophenacil)thio]cytosine, 5-[(4-azidophenacil)thio]uracil, 7-deaza-7-iodoadenosine, 7-deaza-7-iodoguanine, 7-deaza-7-bromoadenosine, and 7-deaza-7- bromoguanine. A "photo-reactive pyrimidine" means every modified pyrimidine capable of crosslinking with a target following irradiation at a certain wavelength. Examples of photo-reactive pyrimidines include 5-bromo-uracil (BrdU), 5-bromo-cytosine (BrdC), 5-iodo-uracil (IdU), and 5-iodo-cytosine (IdC). This can be obtained, for example but not only, through the introduction of modifications to cytidine 2', and uracil (2'-Fluorine, 2'-Amino, and 2'-O-methyl pyrimidine or other), or by capping the ends in different ways, such as but not only, 3'-3' thymidine ligands at end 3', conjugating with PEG or labeling with biotin or fluorochromes of end 5'.

In some forms of embodiment, to bond the aptamer to the vector scaffold biomaterial, the oligonucleotide chain can be provided at end 5', 3' or at both ends, with a functional group such as, but not only, amino groups, sulfhydryl groups, carboxylic groups, azides, vinyl sulphones, acrylics, metacrylics, hydroxylics, phosphorics, maleimidics, N-hydroxysuccinimides, benzoyls, 5-BromoUracil, or 5-IodoUracil.

The bonding of these functional groups to oligonucleotides is routinely performed by commercial companies using known and established methods.

In embodiments according to the present disclosure, the biomaterial can be composed of polyethylenglycole diacrylate (PEGDA). The presence of acrylic groups can create bonds between the monomers, but can also be used for Michael's addition-type reaction and bind certain functional groups such as aminic or thiol groups present on several bioactive compounds, especially if these are proteins. The Michael reaction or Michael addition is the nucleophilic addition of a carbanion or another nucleophile to an α,β-unsaturated carbonyl compound. It belongs to the larger class of conjugate additions.

Addition of an aptamer with adequate functional groups to PEGDA may result in the

spontaneous formation of covalent bonds between the two compounds, so that the aptamer is retained in the hydrogel structure.

According to the present disclosure, a crosslinker can be utilized to bind aptamer to a biomaterial.

According to the present disclosure, functional groups can be bonded to the aptamer directly or can be connected using spacers or crosslinkers, that is, a chain of at least 1 atom of carbon C, optionally from 3 to 18 atoms of carbon C, although the length can be greater or smaller, inserted between the aptamer and the functional group with which the latter is bonded with the biomaterial.

Crosslinking is the process of chemically joining two or more molecules by a covalent bond. The term "crosslinker", or crosslinking reagent, in this description refers to molecules which contain two or more reactive groups or ends capable of chemically bonding with specific functional groups (primary amines, sulfhydryl groups, etc.), on molecules, in this case the biomaterial and the aptamers of the present disclosure.

The chemical nature of the crosslinker depends on the functional groups present on the components. The crosslinking is performed using various known techniques, which depend on the nature of the functional groups present. Non-limiting example of crosslinking between a thiol-functionalized aptamer and a amin-containing biomaterial can be performed as follows. The aptamer is dissolved in Phosphate buffered saline (pH 7.2) at 0.1 mM. The crosslinker, such as but not limited to Succinimidyl-([N-maleimidopropionamido]-ethylenglycol)ester, is added to the dissolved aptamer at 1 mM final concentration. The reaction mixture is incubated for 30 minutes at room temperature or 2 hours at 4°C. The excess crosslinker can be removed using a desalting column equilibrated with Phosphate buffered saline. The thiol-aptamer and desalted crosslinker are combined and mixed in a 1:1 molar ratio or in a molar ratio corresponding to that desired for the final conjugate and consistent with the relative number of sulfhydryl and activated amines that exist on the two molecules. The reaction mixture is incubated at room temperature for 30 minutes or 2 hours at 4°C.

Useful crosslinkers can also be obtained from commercial sources such as Molecular Biosciences Inc. (Boulder, CO, USA), or Thermo Pierce (Rockford, IL, USA) synthesized according to the procedures described in Toki et al (Toki, Cerveny et al. 2002); or in the documents U.S. Pat. N. 6214345, WO 02/088172, U.S. Pat. N.

2003130189, WO 03/026577 and WO 04/032828.

In embodiments according to the present disclosure, the aptamer(s) can contain a thiol group or can be functionalized with a thiol group, or a thiol group is created by reducing a disulfide bond, such as thiolated hyaluoronic acid. A crosslinker carrying a functional group or groups capable to bind thiol groups such as but not limited to maleimide or acrylate groups may be used.

Some non-restrictive examples of crosslinkers for thiol are: Bis maleimide ethane, Bis maleimide butane, Bis maleimide hexane, Tris(2-maleimide ethylamine, N-alpha-Maleimide acetoxy succinimide ester, N-beta-Maleimide propyl-oxysuccinimide ester, N-gamma-Maleimidobutyryloxy succinimide ester, m-Maleimide benzoyl-N-hydroxysuccinimide ester, Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, N-epsilon-Malemidocaproyl-oxysuccinimide ester, Succinimidyl 4-(p-maleimidophenyl)butyrate, Succinimidyl 6-[(beta- maleimidopropionamide)hexanoate], Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxy-(6-amidocaproate), N-kappa-Maleimide undecan oil-oxysulpho succinimide ester.

In embodiments according to the present disclosure, the aptamer(s) can contain aminic group or can be functionalized with an aminic group. A crosslinker carrying a functional group or groups capable to bind aminic groups such as but not limited to acrylate, epoxy groups or Succinimidyl ester groups may be used.

Some non-restrictive examples of crosslinkers for amino groups are: Dimethyl adipimidate-2HCl, Dimethyl pimelimidate-2HCl, Dimethyl suberimidate-2HCl, Dimethyl 3,3'-dithiobispropionimidate-2HCl, 1,5-Difluoro-2,4-dinitrobenzene, Bis(succinimidyl) penta(ethylene glycol), Bis(sulphosuccinimidyl) suberate, Bis[2-(succinimidoxycarbonyloxy)ethyl]sulfone, Disuccinimidyl glutarate, Dithiobis(succinimidylpropionate), Disuccinimidyl suberate, Disuccinimidyl tartrate, 3,3'-Dithiobis(sulphosuccinimidylpropionate), Ethylene glycol bis(succinimidylsuccinate), Ethylene glycol bis(sulphosuccinimidylsuccinate), Tris-succinimidyl aminotriacetate, Dicycloesilcarbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, N-hydroxysuccinimide, Sulfosuccinimidyl(4-iodoacetyl)aminobenzoate, Succinimidyl(4-iodoacetyl)aminobenzoate, Succinimidyl 3-(bromoacetamide) propionate, Succinimidyl iodoacetate, 2-pyridyldithio-tetraoxa octatriacontan-N-hydroxysuccinimide, 2-pyridyldithio-tetraoxa octatriadecan-N-hydroxysuccinimide, Sulfosuccinimidyl 6-[3'-(2-

pyridyldithio)propionamide]hexanoate, Succinimidyl 6-[3(2-pyridyldithio - propionamide] hexanoate, Succinimidyl 3-(2-pyridyldithio)propionate, 4-Succinimidyl Oxycarbonyl-alpha-methyl- alfa(2-pyridyldithio) toluene, Sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxy-(6-amidocaproate), N-epsilon-Malemidocaproil-oxysuccinimide ester, N-gamma-Maleimidobutyryl-oxysuccinimide ester, N-kappa-Maleimidoundecanoil-oxysulfosuccinimide ester, m-Maleimidobenzoyl-N-hydroxysulfosuccinimide ester, Succinimidyl 4-(p-maleimidophenyl)butyrate, N-alpha-Maleimide acetoxy succinimide ester, N-beta- Maleimidopropyloxy succinimide ester, Succinimidyl 6-[(beta-maleimidopropionamide)hexanoate].

In embodiments according to the present disclosure, crosslinkers can include enzyme cleavable sequences for the release of the therapeutic agent. Enzyme cleavable sequences can comprise amino acid sequences recognized and cleaved by membrane bound and/or cell-secreted peptidases, which are peptide-cleaving enzymes well known in the art to recognize particular amino acid sequences and to cleave said sequences between specific amino acids. Such enzymes include, for example and without limitation, matrix metalloproteinases or "MMP's" (also referred to herein as matrixins), e.g., MMP-2, MMP-9, MMP-14, serine proteases, cysteine proteases, elastase, stromelysins, human collagenases, cathepsins, granzymes, dipeptidyl peptidases, plasmins, plasminogen activators, lysozymes and e.g., aminopeptidase P, aminopeptidase A, and aminopeptidase N. Peptides with suitable MMP substrate selectivity include, for example and without limitation, those having the amino acid sequences reported in U.S. Pat. N. 6844318 or publications by Hatakeyama, Akita et al.; Dettin, Muncan et al. 2011; Fonseca, Bidarra et al. 2011; Jang, Kim et al. 2011; van Duijnhoven, Robillard et al. 2011.

The crosslinker can further comprise acid labile bonds, photolabile bonds, peptidase labile bonds and a combination thereof.

In embodiments according to the present disclosure, crosslinkers can have a dendritic structure, i.e. may be a dendritic type, for the covalent union or attachment of one or more aptamers or biomaterials, or more than one drug moiety, through a branching and multifunctional structure or multifunctional linker moiety to the biomaterial (Sun, Wirsching et al. 2002), (Sun, Wirsching et al. 2003), (Paleos, Tsiourvas et al. 2007),

(King, Dubowchik et al. 2002). Dendritic crosslinkers can increase the molar ratio between biomaterial and aptamers, i.e. the molar ratio of aptamers bound to biomaterials. In this way, even if an aptamer has only one functional group, a multitude of aptamers can be attached to the biomaterial through a single crosslinker molecule.

In embodiments according to the present disclosure, crosslinkers can comprise, consist of or essentially consist of a peptide, thus comprising one or more amino acid units. Peptide linker reagents may be prepared by solid phase or liquid phase synthesis methods (Bodanszky 1984) that are well known in the field of peptide chemistry, including t-BOC chemistry (Geiser 1988) and Fmoc/HBTU chemistry (Fields and Noble 1990), on an automated synthesizer such as the Rainin Symphony Peptide Synthesizer (Protein Technologies, Inc., Tucson, Ariz.), or Model 433 (Applied Biosystems, Foster City, CA, US).

Fig. 3 is used to describe example forms of embodiment of a bond between aptamer and biomaterial. The aptamer, for example, can have a sulfhydryl group (-SH), while the biomaterial has an acrylic group. For example, the bond reaction is a Michael addition reaction, which occurs spontaneously in suitable pH conditions and at ambient temperature, by means of which reaction a very stable thioter bond is formed between the two molecules.

The aptamer can be bonded by means of spacers or crosslinkers, e.g. as defined in the present disclosure, to the biomaterial using end 5', 3' or both. The end not bonded to the biomaterial, if present, can be free, it may or may not have capping, or it can be bonded to an accessory molecule, directly or by means of spacers. The accessory molecule can have various nature and consist of, but not only, biotin, a fluorophore, an oligopeptide, a polypeptide with enzyme and/or signal and/or adhesive activity or other molecule configured to increase the efficacy of the aptamer, to confer accessory biological properties, to improve the resistance of the aptamer to the action of enzymatic degradation or to verify and control the quality of the material.

Fig. 4 is used to describe example forms of embodiment of the configuration of the relation between aptamer and functional group, in this case bonded to end 3'. The functional group can be bonded directly to the aptamer or by means of a spacer. An accessory molecule M, such as for example a peptide, can be complexed to the aptamer.

In possible implementations, a molecule of a peptide nature can also be inserted between aptamer and biomaterial with or without spacers before, after or at both ends of

the peptide.

In example forms of embodiment, the peptide can include sequences cleaved from enzymes, to release the bonded molecules. Sequences cleavable from enzymes comprise sequences of amino acids recognized and cleaved from membrane or secreted peptidases, which are enzymes that split known peptides at known points of the sequence. Such enzymes include for example matrix metalloproteinase or "MMP", serine protease, cysteine protease, elastase, stromelysin, human collagenese, catepsin, granzymes, dipeptidyl peptidase, plasmin, plasminogen activators, lysozyme and for example aminopeptidase P, aminopeptidase A and aminopeptidase N. Peptides with substrate selectivity for MMP include for example those described above. These protease sequences can also be present in the scaffold itself, irrespective of the aptamer component. Crosslinkers can also include ligands splittable from acids, photolabiles, or a combination thereof.

In some forms of embodiment, the aptamers can be bonded to the biomaterial through the presence of favorable functional groups on the biomaterial itself, which will be chosen among those known in the state of the art of chemical science, such as but not only: amino groups, sulfhydryl groups, carboxylic groups, azides, vinyl sulfones, acrylates, methacrylics, hydroxylics, phosphorics, maleimides, N-hydroxysuccinimides, benzoyls.

Some biomaterials like hydrogels based on polyethylene glycol diacrylate (PEGDA) consist of a monomer containing due to its very nature a functional group (acrylate) necessary for the polymerization of the monomer which can be used to bond the aptamer supplied by an accessory functional group, for example amino or sulfhydryl.

Some materials, like organic polymers, can be routinely modified following the methods normally used by persons of skill. Other materials, such as those of an inorganic type, such as hydroxyapatite or titanium and metals, do not possess organic functional groups, which must therefore be supplied using state of the art methods. To achieve this purpose it is possible to coat the biomaterial with a coating using standard methods such as sol-gel, sputter coating, plasma spray, to create an intermediate layer on which the aptamers can then be bonded.

Fig. 5 is used to describe example forms of embodiment of the configuration pattern of the aptamer-biomaterial relation: the aptamer can have one free end, be capped, have functional groups at both ends or can be conjugated with an accessory molecule.

In some forms of embodiment it is also possible to combine several biomaterials, to form a composite material, the components of which can both be functionalized to be coated with aptamers or only one of the two, the more manageable one from the chemical point of view. It is therefore possible to propose for example a PEGDA hydrogel functionalized with aptamers, a natural or synthetic hydroxyapatite functionalized with aptamers, the union of the two or the union of a gel functionalized with granules of non-functionalized hydroxyapatite.

In some forms of embodiment, the bonding of an aptamer to a biomaterial is achieved by joining two aptamers, of which one recognizes a protein or another constituent component of the biomaterial, such as but not restrictively collagen, fibronectin, fibrinogen or synthesized molecules, and one remains free to perform its biomimetic function by means of the bond with plasma or tissue or exogen proteins after implantation in the receiving organism. This allows the surface enrichment of fresh tissues, or in any case tissues not processed with the addition of functional groups if these were not present, also chair- or bedside.

The aptamer may be present only on the surface of the biomaterial or in the whole thickness thereof. For example, where the biomaterial has a transition temperature between liquid and solid phase in its final use that is compatible with maintaining the integrity of the aptamer, which could be the case of a hydrogel that is polymerized before the insertion into the wound or simultaneously therewith, the aptamer can be mixed with the monomer, or functionalized on the monomer before polymerization and therefore be present in the thickness of the material when polymerization is complete. This cannot reasonably be done with a metal, the external surface of which will be the only one accessible for enrichment with aptamers.

Aptamers can be added to the biomaterial immediately before insertion into the organism, prior to or after insertion. If the biomaterial is in monomeric form, the aptamer can be supplied already bonded with the monomer to be polymerized or separately, to be added during or after polymerization.

The biomaterials can be entirely functionalized with the same aptamer against a single target or with a mixture of two or more types of aptamer with the same or different chemical nature against the same or different targets. In this case, the two or more aptamers can be present in equal proportions or in different proportions, mixed or distributed differently inside the biomaterial. The distribution may be random or can

follow a development obligated by particular functional requirements of the material. Different portions of the biomaterial or medical device or prosthesis can be coated with different aptamers or with aptamers against different targets. If the material or medical device is composite, the different components will be able to have different aptamers or aptamers against different targets or different proportions of aptamers.

The biomaterial-aptamer compound may possibly be enriched by one or more additional molecules, both targets or not targets of the aptamer, with a pharmacological function, such as, but not limited to, antibiotics, inhibitors, agonists or growth factors. Such additional molecule or molecules can be added to the material, adsorbed on its surface if in solid form, mixed with it if in liquid or gel form, they can be bonded with the material itself by means of a covalent bond, in order to be retained on the material or released into the surrounding tissue.

According to the embodiments described herein, an aptamer can be bound to biomaterials having metallic nature, such as endosseous dental or orthopedic implants or bioceramic-based bone substitute tissue. In particular, an aptamer according to the present disclosure can be bound covalently with an organic residue. Residues made of separate monomers which can be the same or different and which have aromatic side chains that are directly adjacent or separated by one or more monomers are usable as organic residues of the present invention. Examples for such organic residues are such with backbone structure, herein simply referred to as backbone, having C atoms, like polymers selected from ethylene, propylene, amides, ester-, ether- or thioester compounds which have aromatic side chains, such as phenyl- or naphthylgroups, heterocycles, aromatic amino acids, like Phenylalanine (Phe), Tyrosine (Tyr) or Trpyptophane (Trp) etc and which carry in addition at least one hydroxyl group at the organic residue. Backbones can be linear or branched polymers. Exemplarily, at least partly hydroxylated polystyrene or amino acid sequences having aromatic hydroxyl substituted amino acids are mentioned. Alternatively, silicon based polymers such as siloxanes or silanes can be used. The number of monomers can be up to about 50 wherein at least more than about 5 hydroxyl groups should be present in the organic residue to provide for a sufficient interaction with the surface.

As a result of the hydroxyl groups present at the aromatic side chain, the interactions between the organic residue and the surface of the material have a high stability, thus enabling a similarly stable coating. In this way, it is possible to link the aptamer as

previously described via the organic residue on the substrate surface and thus make it usable for further applications, wherein the aptamer binds targets such as extracellular matrix proteins, growth factors such as PDGF, BMPs, or VEGF as well as possible bioactive molecules, factors or tissue hormones, as described above.

Thereby, the organic residue can be bound at the 5' or 3' end of the aptamer, or the organic residue having aromatic side chains can be interposed into the aptamer as long as the binding affinity of the aptamer to its target is not affected adversely, or multiple aptamers can be bound to the side chains or extremities of the organic residue.

At the same time it is especially preferred that at least one aromatic side chain of the organic residue is hydroxylated chemically or enzymatically so that this aromatic side chain carries two hydroxyl groups, to enhance the interaction with the surface of a substrate, in particular with such substrates carrying an oxy or hydroxy group, which have a surface made out of metal oxide, metal hydroxide, calcium hydroxyphosphonate (hydroxyapatite), silicium oxide or -hydroxide as it can be found with metals, ceramics or glasses.

In an alternative embodiment of the method, in a first step an aptamer is covalently bound to an organic residue which comprises a backbone structure with aromatic side chains, and a modified aptamer made of an aptamer and an organic residue having aromatic side chains is formed, having the following structure:

A—[Ar1—(X)n]w —[Ar2—(X)n]y — [Ar3—(X)n]z

wherein:

A represents the aptamer which is linked through its 5' or 3' end with the organic residue having aromatic side chains or in which the organic residue having aromatic side chains is interposed;

Ar1, Ar2 and Ar3 are the same or different and each represents an aromatic amino acid which is selected from the group consisting of tyrosine, tryptophane phenylalanine, or an unnatural or synthetic aminoacid presenting at least one aromatic sidechain group;

X represents any amino acid which is the same or different within the units [Ar1—(X)n]w, [Ar2—(X)n]y and [Ar3—(X)n]z;

n is 0 to 10 inclusively;

w, y and z represent a natural number from about 0 to 50; and

in a second step at least one of Ar1, Ar2 and Ar3 is modified chemically or enzymatically in such a way that at least two hydroxyl groups are present at the

aromatic ring.

As used herein, term "amino acid," in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain.

In some embodiments, an amino acid has the general structure H2N—C(H)(R)—COOH.

In some embodiments, an amino acid is a naturally-occurring amino acid.

In some embodiments, an amino acid is an unnatural amino acid.

In some embodiments, an amino acid is a synthetic amino acid.

In some embodiments, an amino acid is a D-amino acid.

In some embodiments, an amino acid is a L-amino acid.

As used herein, "natural amino acid" refers to any of the twenty standard L-amino acids commonly found in naturally-occurring peptides: Histidine, Alanine, Isoleucine, Arginine, Leucine, Asparagine, Lysine, Aspartic acid, Methionine, Cysteine, Phenylalanine, Glutamic acid, Threonine, Glutamine, Tryptophan, Glycine, Valine, Ornithine, Proline, Serine, Tyrosine.

As used herein, "unnatural amino acid" encompasses any amino acid other than the 20 natural amino acids, such as hydroxyphenylglycine. Unnatural amino acids may be chemically produced or modified amino acids, including but not limited to salts and/or amino acid derivatives (such as amides). Amino acids, including carboxy- and/or amino-terminal amino acids in peptides, can be modified by methylation, amidation, acetylation, and/or substitution with other chemical groups. Amino acids may participate in a disulfide bond.

The natural aromatic amino acids are L-phenylalanine (Phe), L-tyrosine (Tyr) and L-tryptophane (Trp) or their destro isomers D-phenylalanine, D-tyrosine and D-tryptophane. Genetically producible organic amino acid sequences which can be used according to the present invention might consist of n=1-50 aromatic amino acid sequences, like —(Phe)n—, —(Tyr)n—, —(Trp)n— or combinations thereof, which are bound N-terminal or C-terminal at the A aptamer. For X, these amino acid sequences can comprise any amino acid which is the same or different within the units [Ar1—(X)n]w, [Ar2—(X)n]y and [Ar3—(X)n]z. Thereby, also such analog compounds are included in which the stereo chemistry of the separate amino acids is changed in one or more specific positions from L/S to D/R. Also included are analog compounds which possess a peptide character only to a lesser extent. Such peptide mimetics can comprise

for example one or more of the groups of the following substitutions for CO—NH-amid linkages: depsipeptide (CO—O), iminomethylene (CH2—NH), trans-alkene (CH=CH), enaminonitrile (C(=CH—CN)—NH), thioamide (CS—NH), thiomethylene (S—CH2), methylene (CH2—CH2) and retro-amide (NH—CO) which, for example, increase the stability of the organic residue [Ar1—(X)n]w, [Ar2—(X)n]y and [Ar3—(X)n]z compared to proteases in a physiological environment. These substitutions can be used within the organic residue of the invention at every spot where peptide linkages can be found.

Hydroxylation of the aromatic side chains can be performed by chemical procedures or enzymatically known to those skilled in the art. Therefore, in the method according to the present disclosure it is preferred that at least one aromatic residue is hydroxylated chemically or enzymatically so that two hydroxyl groups are present adjacent at the aromatic ring, more preferably three hydroxyl groups are present adjacent at the aromatic ring.

In one embodiment of the invention n is smaller than three, preferably equal 0 or 1, and w , y and z are each an integral number from about 1 to 5 in the above formula [Ar1—(X)n]w, [Ar2—(X)n]y and [Ar3—(X)n]z.

Thus, the present disclosure further refers to an aptamer modified with an organic residue, which is formed out of an aptamer and an organic residue having aromatic side chains, wherein at least one aromatic side chain of the organic residue is hydroxylated chemically or enzymatically.

In this way, the organic residue of the aptamer has preferably two hydroxyl groups in at least one aromatic side chain.

Of particular importance as organic residues are poly-phe, poly-tyr and poly-trp, which can interact for example on a metallic surface directly via n-n or d-n donor-acceptor interactions with corresponding n- or d-electron containing compounds due to their aromatic character. Furthermore, they can be converted in corresponding hydroxy compounds after introducing hydroxyl groups by means of oxidation processes. For example, tyrosine is a natural aromatic hydroxy compound. For example, by introducing another hydroxyl group in tyrosine dihydroxyphenylalanine is formed and out of a corresponding poly-tyr (-(tyr)n-) a poly-DOPA (-(DOPA)n-) is formed.

Using a method of coating a substrate allows applying a solution of an aptamer-organic residue on the surface of a substrate and immobilizing the aptamer-organic

residue via covalent or non-covalent interaction on the surface of the substrate. In particular, the substrate can be made of metal, ceramic or glass and have a surface made of metal oxide, metal hydroxyide, calcium hydroxyphosphonate (hydroxyapatite), silicium oxide or -hydroxide carrying oxy- or hydroxy groups.

In case of poly-tyr, the poly-tyrosine-tag is already present as polyphenolic group after the first step and can be used directly for a binding reaction, e.g., on metal surfaces. However, it can be expected that introducing a second phenolic hydroxyl group in tyrosine leads to an increase of binding specificity and affinity and thus binding energy. Thus, in a second step one or more phenolic hydroxyl groups can be introduced into the aromatic ring system of phenylalanine, tyrosine or tryptophane. Following this way, the amino acid tyrosine (4-hydroxy-phenylalanine) can be transferred, for example, to 3,4-dihydroxyphenylalanine (DOPA). Thus, a poly-DOPA-tag can be produced out of a poly-tyrosine-tag. A further hydroxylation to 3,4,5-trihydroxyphenylalanine (TOPA) is also possible. The polyphenolic tag, e.g. poly-DOPA-tag, can then confer to specific adhesion properties on metal surfaces, in particular transition metals, glass surfaces or ceramics, so that a permanent coating of the surface material can be provided for varied biological, chemical and medical applications.

Polyphenolic tags, such as poly-DOPA, can undergo specific binding reactions with certain transition metal oxides on metal surfaces. The following chemical reaction types for binding of an aptamer via a poly-DOPA-tag to a titanium surface may occur:

1. Ionic interactions between positive charges on the titanium surface and negative charged phenolate ions of poly-DOPA.

2. Electron-donor-acceptor complex in the form of a d-n interaction between titanium (d-orbital) and the n-electrons of the phenolic ring.

3. It might also be possible that a direct metal-organic linkage between DOPA and the titanium surface is assembled.

The specific adhesion properties, for example of poly-DOPA-tags to aptamers are used in the method of the present invention to directly immobilize aptamers selectively and with high affinity on metal- or glass surfaces. Presumably, the hydroxyl groups in ortho position at the phenyl residue (i.e. DOPA) of the (DOPA)3 structure are responsible for the high affinity binding of residual-DOPA at the hydroxyl groups of a titanium dioxide surface which can be found on metallic titanium. By simultaneous

reaction of several DOPA-residues in a poly-DOPA molecule with the titanium surface, the affinity of the bond will increase in a power function so that extremely high binding affinities can be reached. Transition metal oxide containing surfaces can be transformed to support materials for aptamers carrying organic residues and can be used for synthesis of biological active surfaces in the area of tissue engineering and biomaterial engineering. Application of this technology is also possible on glass surfaces. Thus, matrices for aptamers that bind any target molecule can be prepared, which can also prove of value in the area of chromatography, immunoassays and array technology.

The single or multiple hydroxylated aromatic poly-amino acids which are covalently bound with an aptamer serve as anchor structure for the tight linkage of the aptamer to a silicium oxide- or metal oxide containing matrix. Through this bond to the oxide containing matrix, the aptamer-organic residue can be immobilized in a biological active form on a metal surface, for example of a titanium implant.

Furthermore, it is also possible herein, as previously described above, that homo- or heteropolymers of the precursor tyrosine are present which can be transformed into the corresponding homo- or heteropolymers of DOPA afterwards. In this case it could be of particular importance that the DOPA molecules maintain a defined distance within the polypeptide which corresponds to the specific steric proportion of the metal oxide layer of the metal surface. In a similar way, poly anorganic amino acid hybrids can be prepared based on the amino acids phenylalanine and tryptophane.

According to a third possibility, A—[Ar1—(X)n]w—[Ar2—(X)n]y—[Ar3—(X)n]z, such as poly-X sequences, can also be interposed in an aptamer as long as the biological activity allows it.

According to embodiments described herein, the biomaterial can comprise viral particles. Viral particles that can be used include but are not limited to retrovirus, lentivirus, adeno-associated virus, poxvirus, alphavirus, baculovirus, vaccinia virus, herpes virus, Epstein-Barr virus, and adenovirus vectors, as well as any combination thereof.

Such viruses can be engineered using the techniques known to those skilled in the art and can carry vectors that include useful genes for tissue regeneration. In addition to a nucleic acid of interest, a vector may also comprise one or more regulatory regions (e.g., promoters, enhancers, termination sequences, etc.), and/or selectable markers useful in selecting, measuring, and monitoring nucleic acid transfer results (delivery to specific

tissues, duration of expression, etc.).

"Promoter" refers to a nucleic acid sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. Promoters may be derived in their entirety from a native sequence, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleic acid segments. It is understood by those skilled in the art that different promoters may direct the expression of a nucleotide sequence in different tissues or cell types and/or at different stages of development and/or in response to different environmental or physiological conditions.

Promoters that cause a nucleotide sequence to be expressed in most cell types at most times are commonly referred to as "constitutive promoters."

Promoters that cause a nucleotide sequence to be expressed in a specific cell type are commonly referred to as "cell-specific promoters" or "tissue-specific promoters."

Promoters that cause a nucleotide sequence to be expressed at a specific stage of development or cell differentiation are commonly referred to as "developmentally-specific promoters" or "cell differentiation-specific promoters."

Promoters that are induced and cause a nucleotide sequence to be expressed following exposure or treatment of the cell with an agent, biological molecule, chemical, ligand, light, or the like that induces the promoter are commonly referred to as "inducible promoters" or "regulatable promoters." It is further recognized that, because in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleotide sequences of different lengths may have identical promoter activity.

According to embodiments described herein, the biomaterial can comprise nanoparticles, that is particles smaller than 1 micron in size. The term “nanoparticle” may include a wide range of structures and constructs such as polymeric micelles, dendrimers, polymeric, metal and ceramic nanoparticles, protein cage architectures, viral-derived capsid nanoparticles, DNA nanoparticles, polyplexes, solid lipid particles and liposomes (J Hereen 2006).

According to embodiments described herein, the biomaterial can comprise liposomes. As used herein, the term “liposome” refers to a self-assembling structure comprising one or more lipid bilayers, each of which comprises two monolayers containing oppositely oriented amphipathic lipid molecules. Amphipathic lipids comprise a polar (hydrophilic) headgroup covalently linked to one or two or more non-

polar (hydrophobic) acyl or alkyl chains. Energetically unfavorable contacts between the hydrophobic acyl chains and a surrounding aqueous medium induce amphipathic lipid molecules to arrange themselves such that polar headgroups are oriented towards the bilayer's surface and acyl chains are oriented towards the interior of the bilayer, effectively shielding the acyl chains from contact with the aqueous environment.

Liposomes useful in connection with the methods and compositions described herein can have a single lipid bilayer (unilamellar liposomes) or multiple lipid bilayers (multilamellar liposomes) surrounding or encapsulating an aqueous compartment. Various types of liposomes are described, e.g., in Cullis et al., Biochim. Biophys Acta, 559: 399-420 (1987).

Amphipathic lipids typically comprise the primary structural element of liposomal lipid vesicles. Hydrophilic characteristics of lipids derive from the presence of phosphate, carboxylic, sulphate, amino, sulfhydryl, nitro, and other like polar groups. Hydrophobicity can be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups, which may be substituted by one or more aromatic, cycloaliphatic or heterocyclic group(s). Non-limiting examples of preferred amphipathic compounds are phosphoglycerides and sphingolipids, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, phoasphatidylglycerol, palmitoyloleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidylethanolamine, dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine, distearoylphosphatidylcholine (DSPC), dilinoleoylphosphatidylcholine and egg sphingomyelin.

Other lipids such as sphingolipids and glycosphingolipids, are also useful in methods and compositions provided herein. Additionally, the amphipathic lipids described above may be mixed with other lipids, such as triacylglycerols and sterols.

The nanoparticles used according to the present disclosure may be formed with any technologies among those known to those skilled in the art to which they pertain. Nanoparticles can be functionalized with peptides, proteins, nucleic acids or other biologically active compounds.

EXPERIMENTAL DATA

We first proceeded to test the hypothesis that aptamers could improve cell binding to

biocompatible scaffolds. To this purpose, we had anti-human Fibronectin DNA aptamers screened for (Basepair Biotechnology, Houston, TX) and functionalized with biotin on their 5' end and a short carbon chain containing a S-S bond on their 3' end. The S-S bonds were reduced with TCEP prior to use, so that they yielded free thiol-groups, freshly before use. We then selected a 3D matrix which could be easily enriched with aptamers, such as Polyethylene glycol -based hydrogels (PEG). These hydrogels possess functional groups that can be used to easily bind organic molecules such as aptamers and are often used to culture cells after addition of adhesion molecules, because PEG alone offer scant adhesion to cells, and it has been proposed as an appropriate scaffold for stem cells, because it does not promote cell differentiation through cell adhesion. PEG gels therefore appeared as a promising substrate to test our hypothesis.

Aptamers were mixed to Polyethylene glycol diacrylate (PEGDA) and thiolated hyaluronic acid (tHA, Glycosan, Biotime Inc., San Francisco) and allowed to set. The thiol groups on their 3' end bound to PEGDA acrylate groups through a nucleofilic addition (Michael's addition) and thus immobilized aptamers on the matrix. Aptamers were detected by labeling their 5' end with Streptavidin-Alkaline Phosphatase (Figure 6), using a colorimetric assay to detect the amount of substrate converted by the enzyme, thus calibrating the optimal aptamer concentration for subsequent experiments. When only 10% Fetal Bovine Serum was used, little aptamer enrichment was found, but as human serum was added to the culture well, even at very low (0.5%) concentration, significantly more aptamers bound to the hydrogel, indicating a high specificity of these aptamers to human Fibronectin (Figure 6).

Aptamer binding of Fibronectin was also investigated by immunofluorescence, using FITC conjugated anti-Fibronectin antibodies, after addition of human serum to the wells (Figure 7). In particular, Figure 7 shows immunofluyorescent staining of human Fibronectin on hyrdogels in the absence (left panel) or un the presence (right panel) of anti-Fibronectin aptamers, using FITC labelled anti-Fibronectin antibody. Presence of Fibronectin is shown as green fluorescence on gels. Fluorescence intensity was significantly higher on aptamer-enriched gels than on controls.

We further investigated cell adhesion on hydrogel matrices is enhanced by anti-Fibronectin aptamers. To investigate the hypothesis that aptamers-enriched hydrogels could promote cell adhesion, we plated primary human osteoblasts (hOB) on the surface

of set hydrogel in the presence or in the absence of aptamers and added DMEM enriched with 10% human serum in 24 well plates. Cells were cultured for 10 days and medium was changed every third day. At the end of the experimental period gels were rinsed with PBS and cells were fixed and stained. Although few hOB cells were observed on control hydrogel, may cells had attached and proliferated on aptamer-enriched gel, creating little cell clusters that covered the whole surface of the well (Figure 8). In particular, Figure 8 shows cells on aptamer-rich hydrogels after 10 days culture. Cells were fixed and stained with Methylene Blue (Magnification: 10X). Numerous cell clusters can be observed on the surface of the well, in contrast to control hydrogels (inset), where no cells were visible after rinse with PBS.

Cells on aptamer-containing hydrogels could be observed inside the gel, on multiple focus planes, whilst no such thing was encountered in control gels (data not shown). Cells cultured as described above were also fixed and stained with FITC-conjugated anti-Vinculin antibody and DAPI for fluorescent labeling of focal adhesions and nuclei respectively. More cells were observed on aptamer-enriched hydrogels and their cytoplasm appeared more spread and richer in adhesion complexes (Figure 9). In particular, Figure 9 shows immunofluorescence staining of osteoblasti cells on PEGDS-tHA hyrdogels in the absence (left panel) or in the presence (right panel) of anti-Fibronectin aptamer. Magnification: 40X. Blues: Nuclesi, Green: Vinculin, Red: Actin.

We quantitated cell number on Control and aptamer-enriched hydrogels by MTT assay after rinsing (Figure 10). Significantly more cells were observed on PEGDA-tHA hydrogels after addition of aptamers binding to Fibronectin.

We further investigated cell colonization of 3D matrices enhancement by anti-Fibronectin aptamers. We proceeded to investigate whether aptamers could promote cell growth in 3D hydrogel models. To this purpose, hOB cells were encapsulated in PEGDA-tHA hydrogel in the presence or in the absence of anti-Fibronectin aptamers and were cultured in DMEM medium enriched with 10% human serum. After 10 days, gels were fixed, paraffin enclosed and cut. Sections were then stained with hematoxylin-eosin and analyzed at the microscope. Cells were present in both groups, since they had been encapsulated in the matrix. However, beside cells being more numerous in the presence of aptamers (Figure 11), cell morphology appeared quite different: cells were very elongated in the control group and possessed long, sometimes with neurite-like appearance, cytoplasmic extroflections, which can be interpreted as a way to increase

cell adhesion in a less favorable environment. In contrast, cells in aptamer-containing gels had wider cytoplasm with broad podosomes, suggesting a firmer attachment to the substrate. Figure 11 shows in particular hematoxylineosin stained sections from osteoblastic cells encapsulated into PEGDA-tHA hydrogel in the absence (left panel) or in the presence (right panel) of anti-Fibronectin aptamers. Magnification: 20X.

It is clear that modifications and/or additions of parts may be made to the aptamers for making biomedical devices that can be implanted into tissues and corresponding method as described heretofore, without departing from the field and scope of the present invention.

It is also clear that, although the present invention has been described with reference to some specific examples, a person of skill in the art shall certainly be able to achieve many other equivalent forms of aptamers for making biomedical devices that can be implanted into tissues and corresponding method, having the characteristics as set forth in the claims and hence all coming within the field of protection defined thereby.

CLAIMS

1. Biomedical device configured to be implanted in bone or cartilaginous tissue to restore the anatomy or functionality of said bone or cartilaginous tissue, said biomedical device comprising one or more biomaterials coated with aptamers, wherein the biomaterial is chosen from a group comprising: collagen, hydrogel, fresh or processed autologous tissue, processed homologous or heterologous tissue, a fresh or processed tissue extract, demineralized bone matrix, natural apatite, organic sponge, implantable matrix, bioceramics, bioglass, endosseous or orthopedic implant, an implantable prosthesis, a sensor, a biosensor, a metal, a metal alloy or any combination thereof.

2. Biomedical device as in claim 1, wherein one or more aptamers are chosen from a group comprising: single or double stranded D-deoxyribonucleic acid, single or double stranded D-ribonucleic acid, their levo chiral isomer equivalent, oligopeptides.

3. Biomedical device as in claim 1 or 2, wherein one or more aptamers are selected to directly or indirectly bond one or more molecules chosen from:

- target molecules, proteins or not, human or animal molecules of biological interest, intracytoplasmatic or extracytoplasmatic, present in plasma as fibrinogen or albumin or globulin, or in the tissue to be regenerated or in other tissues of the organism with structural function, comprising Collagen, Laminin, Elastin, Fibronectin, Vitronectin, F-spondin, Periostin, Thrombospondin, proteoglycans including Heparan-sulfate, Chondroitin sulfate, Keratan sulfate, Hyaluronic acid, or with signal function, comprising cytokines including IL-1, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-16, IL-17, IL-23, IL-34, IL-35, Tumor necrosis factor (TNF), Interferon, chemokine, including but not only CCL1, CCL2, CCL3, CCL5, CCL6, CCL7, CCL8, CCL9, CCL11, CCL12, CCL13, CCL14, CCL1, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, Osteoprotegerin, RANKL, Sclerostin, DKK, sFRP, immunoglobulin, or growth factors, comprising Adrenomedullin (AM), Angiopoietin (Ang), Autocrine motility factor, Bone morphogenetic proteins (BMPs), Brain-derived neurotrophic factor (BDNF), Epidermal growth factor (EGF), Erythropoietin (EPO), Fibroblast growth factor (FGF), Glial cell line-derived neurotrophic factor (GDNF), Granulocyte colony-stimulating factor (G-CSF), Granulocyte macrophage colony-stimulating factor (GM-CSF), Growth differentiation factor-9 (GDF9), Human growth/differentiation factor-5 (GDF-5), Hepatocyte growth factor (HGF), Hepatoma-derived growth factor (HDGF), Insulin-

like growth factor (IGF), Migration-stimulating factor, Myostatin (GDF-8), Nerve growth factor (NGF), Platelet-derived growth factor (PDGF), Thrombopoietin (TPO), Transforming growth factor (TGF), Vascular endothelial growth factor (VEGF), Wnt family growth factors, Placental growth factor (PlGF), or hormones, comprising parathyroid hormone (PTH), Growth Hormone (GH), Insulin, Calcitriol, Estrogens, Progesterone, Prolactin, Testosterone, or components of the signal cascades;

- nucleic acids, comprising oligonucleotides, Plasmid DNA, fragments of chromosome DNA, RNA, siRNA, shRNA, in which optionally the one or more aptamers are configured to bond proteins which have the function of vectors of said nucleic acids;

- exogen molecules, comprising drugs selected from a group comprising chemotherapy drugs, antibiotics, drugs that promote scar formation, the regeneration of tissue, the functionality of tissue;

- membrane cell receptors for cytokines, growth factors or hormones present on the cells of the tissue, or of other tissues, or exogen cells supplied for the purpose of the therapy and to contribute to the activation of signal ways therein and to the modulation of the cell activity.

4. Biomedical device as in any claim hereinbefore, wherein the one or more aptamers are modified through the introduction of modifications to the nitrogenous bases, to the sugars or to the bonds forming the nucleotides, including modifications to the cytidine 2', and uracil, or through capping of the ends or the introduction of photo-activatable nucleotides.

5. Biomedical device as in any claim hereinbefore, wherein the one or more aptamers are provided with an oligonucleotide chain which has at one 5', 3' end, or at both ends, at least a functional group, bonded directly to the aptamer or by means of spacers, wherein the functional group is chosen from: amino groups, sulfhydryl groups, carboxylic groups, azides, vinyl sulfones, acrylics, methacrylics, hydroxylics, phosphorics, maleimides, N-hydroxysuccinimides, benzoyls, 5-bromoUracil or 5-iodoUracil.

6. Biomedical device as in any claim hereinbefore, wherein one end of the one or more aptamers not bonded to the biomaterial is free, or which has or has not capping, or is bonded to an accessory molecule, either directly or by means of spacers.

7. Biomedical device as in claim 6, wherein said accessory molecule is a molecule configured to increase the efficacy of the aptamer, to confer accessory biological

properties and/or biocompatibility, to improve the resistance of the aptamer to the action of enzymatic degradation or to verify and control the quality of the material.

8. Biomedical device as in claim 6 or 7, wherein said accessory molecule is chosen from a group comprising: biotin, a fluorophore, an oligopeptide, a polypeptide with enzyme activity and/or signal activity and/or adhesive activity.

9. Compound comprising at least one biomaterial and one or more aptamers which at least coat the at least one biomaterial, , wherein the biomaterial is chosen from a group comprising: collagen, hydrogel, fresh or processed autologous tissue, processed homologous or heterologous tissue, a fresh or processed tissue extract, demineralized bone matrix, natural apatite, organic sponge, implantable matrix, bioceramics, bioglass, endosseous or orthopedic implant, an implantable prosthesis, a sensor, a biosensor, a metal, a metal alloy or any combination thereof.

10. Compound as in claim 9, wherein the one or more aptamers are present only on the surface of the biomaterial or the entire thickness of the biomaterial.

11. Compound as in claim 9 or 10, wherein the one or more aptamers are bonded, optionally by means of spacers, to the biomaterial by the 5', 3' end or both.

12. Compound as in claim 9, 10 or 11, wherein the one or more aptamers are bonded to the biomaterial by functional groups provided on said biomaterial and chosen from a group comprising: amino groups, sulfhydryl groups, carboxylic groups, azides, vinyl sulfones, acrylates, hydroxylics, phosphorics, maleimides, N-hydroxysuccinimides, benzoyls.

13. Biomedical device implantable in tissue comprising a compound as in any claim from 9 to 12.

14. Method to manufacture biomedical devices implantable in bone or cartilaginous tissue to restore the anatomy or functionality of said bone or cartilaginous tissue, said method providing to at least coat at least one biomaterial using one or more aptamers, wherein the biomaterial is chosen from a group comprising: collagen, hydrogel, fresh or processed autologous tissue, processed homologous or heterologous tissue, a fresh or processed tissue extract, demineralized bone matrix, natural apatite, organic sponge, implantable matrix, bioceramics, bioglass, endosseous or orthopedic implant, an implantable prosthesis, a sensor, a biosensor, a metal, a metal alloy or any combination thereof.

15. Method as in claim 14, which provides to modify the one or more aptamers

introducing modifications to the nitrogenous bases, to the sugars or to the bonds forming the nucleotides, including modifications to the cytidine 2', and uracil, or through capping of the ends or the introduction of photo-activatable nucleotides and/or to bond, directly or by means of spacers, at one 5', 3' end or at both ends of the oligonucleotide chain of the one or more aptamers, at least a functional group chosen from: amino groups, sulfhydryl groups, carboxylic groups, azides, vinyl sulfones, acrylics, methacrylics, hydroxylics, phosphorics, maleimides, N-hydroxysuccinimides, benzoyls, 5-bromoUracil or 5-iodoUracil.

16. Method as in claim 14 or 15, said method providing to keep free one end of the one or more aptamers not bonded to the biomaterial, or to subject to capping one end of the one or more aptamers, or to bond, directly or by means of spacers, an accessory molecule to one end of the one or more aptamers,

17. Method as in claim 16, wherein said accessory molecule is a molecule configured to increase the efficacy of the aptamer, to confer accessory biological properties and/or biocompatibility, to improve the resistance of the aptamer to the action of enzymatic degradation or to verify and control the quality of the material.

18. Method as in claim 16 or 17, wherein said accessory molecule is chosen from a group comprising: biotin, a fluorophore, an oligopeptide, a polypeptide with enzyme activity and/or signal activity and/or adhesive activity.

19. Method as in any claim from 14 to 18, which provides to bond the one or more aptamers to the biomaterial by means of functional groups provided on said biomaterial and chosen from a group comprising: amino groups, sulfhydryl groups, carboxylic groups, azides, vinyl sulfones, acrylics, hydroxylics, phosphorics, maleimides, N-hydroxysuccinimides, benzoyls.

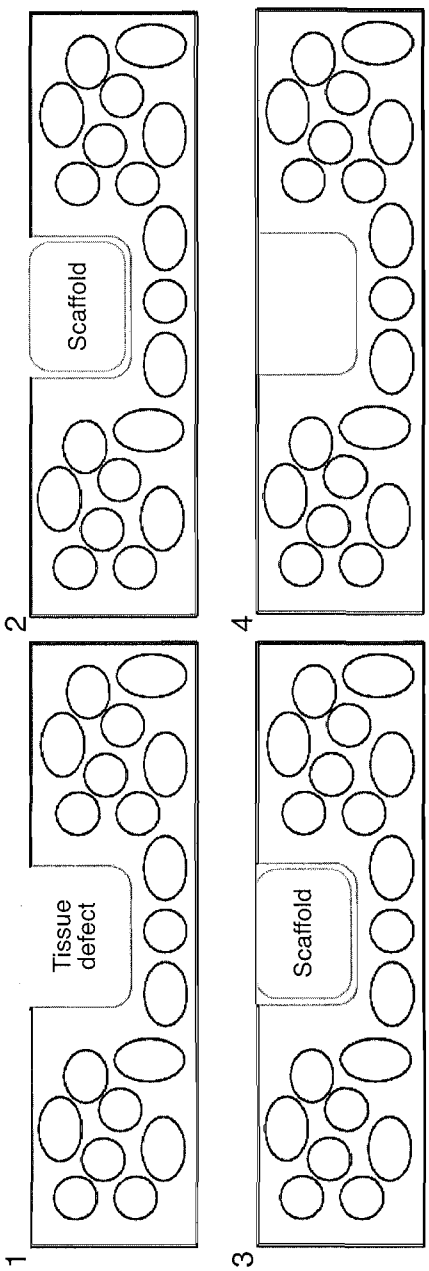
1
Tissue defect
2
Scaffold
3
Scaffold
4

fig. 1

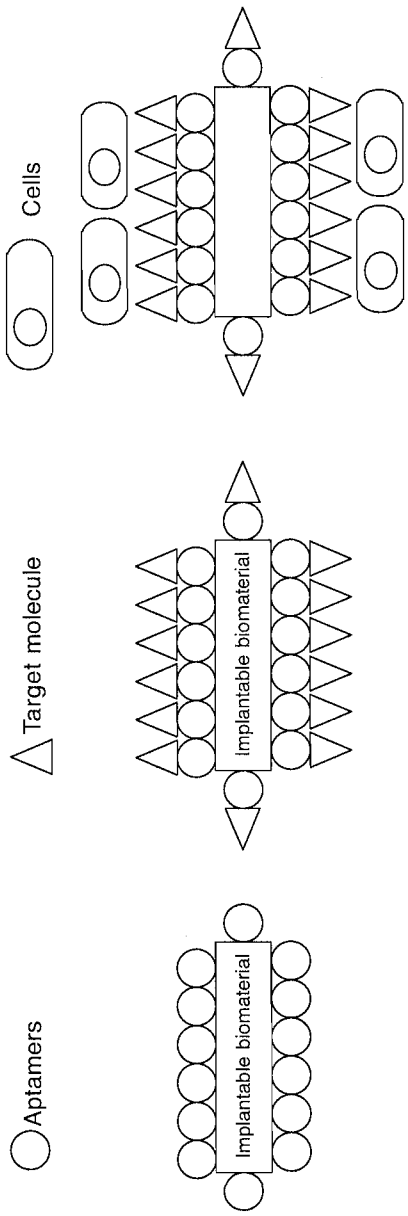
Aptamers
Target molecule
Cells
Implantable biomaterial
Implantable biomaterial

fig. 2

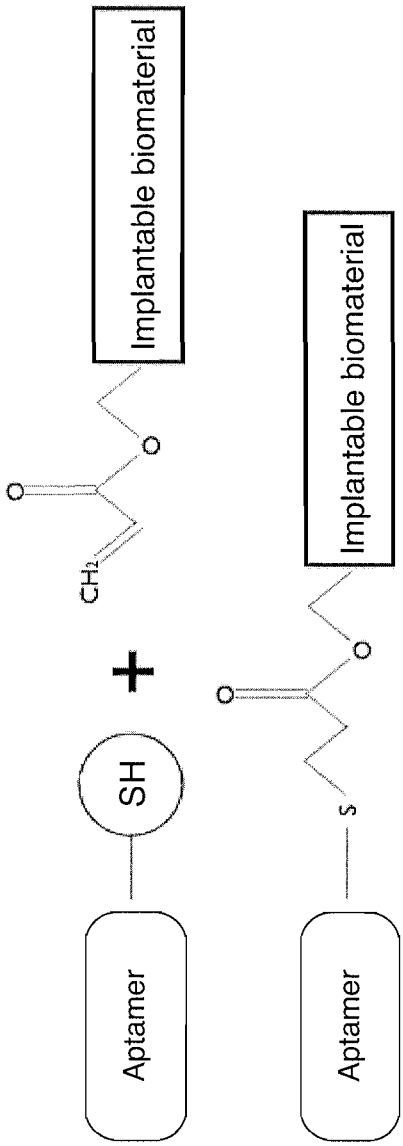
Implantable biomaterial
O
O
CH2
+
SH
Aptamer
Implantable biomaterial
O
O
S
Aptamer

fig. 3

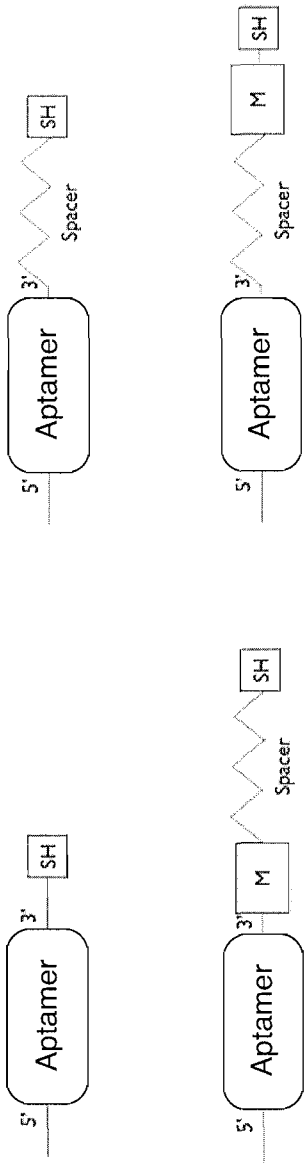
SH
3'
Spacer
Aptamer
5'
SH
M
3'
Spacer
Aptamer
5'
SH
3'
Aptamer
5'
SH
M
Spacer
3'
Aptamer
5'

fig. 4

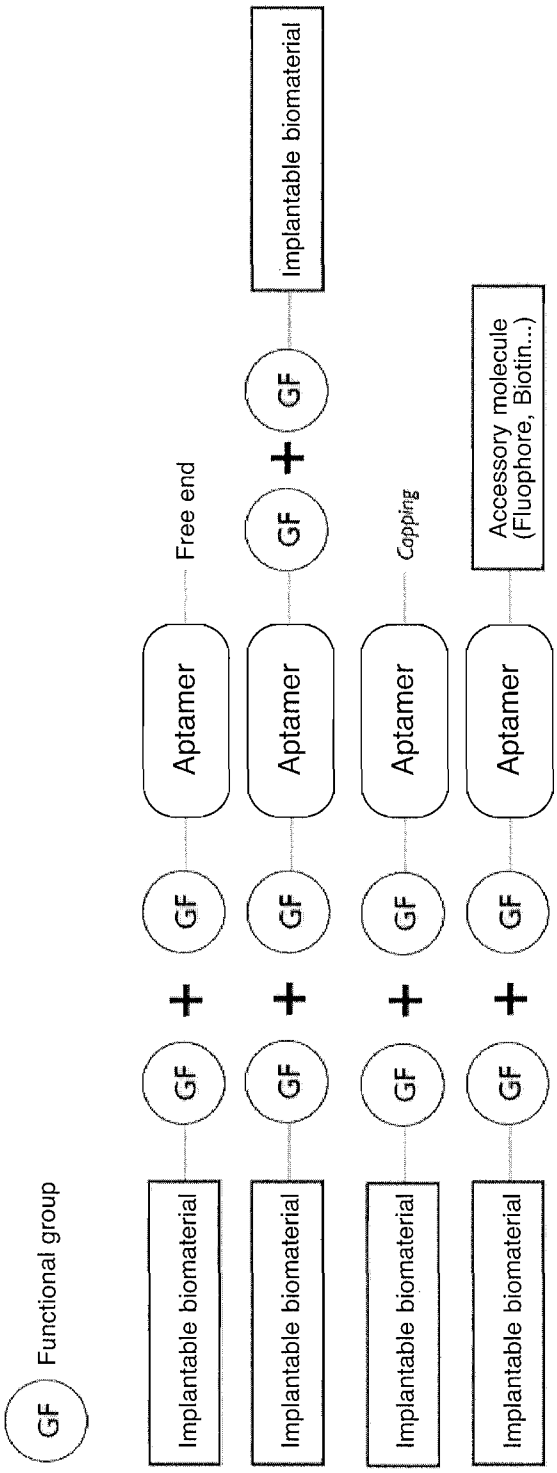
GF Functional group
Implantable biomaterial
GF
+
GF
Aptamer
Free end
Implantable biomaterial
GF
+
GF
Aptamer
Implantable biomaterial
GF
+
GF
Aptamer
Capping
Implantable biomaterial
GF
+
GF
Aptamer
Accessory molecule (Fluophore, Biotin...)

fig. 5

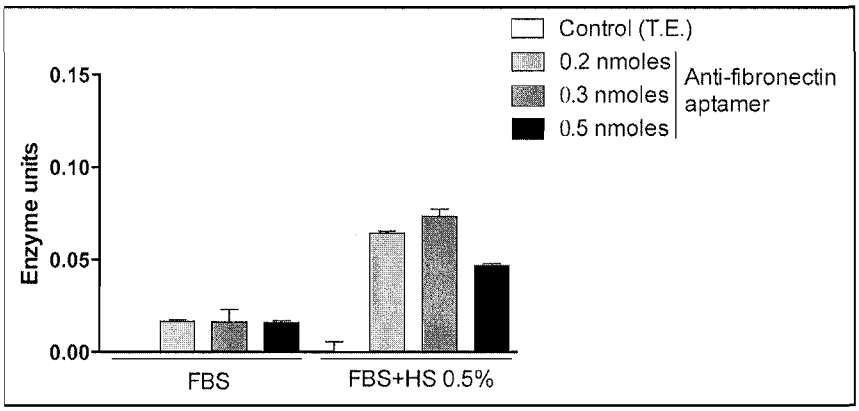

fig. 6

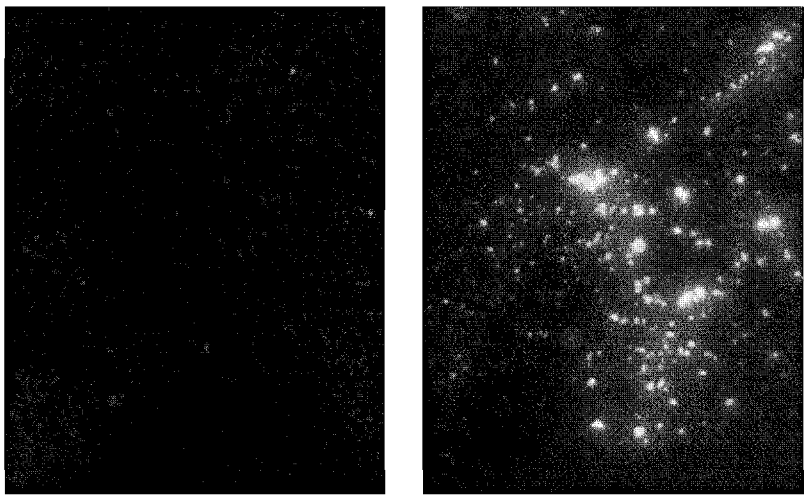

fig. 7

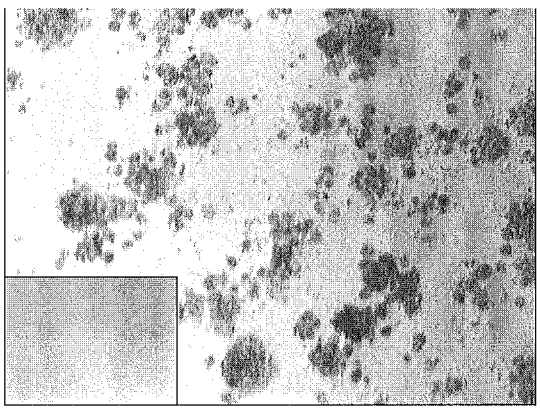

fig. 8

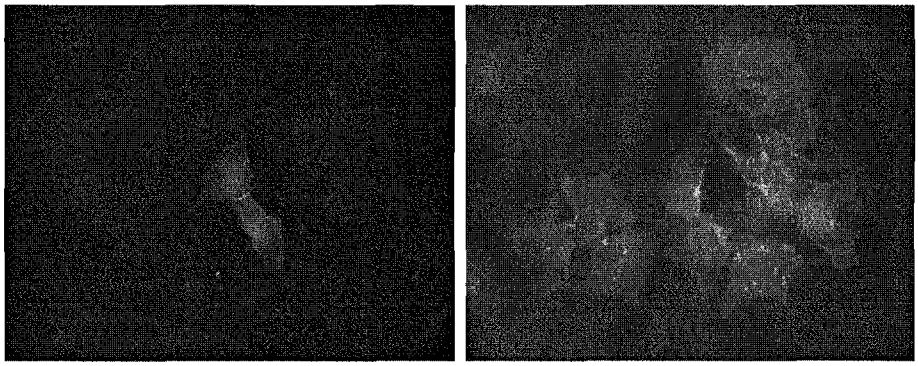

fig. 9

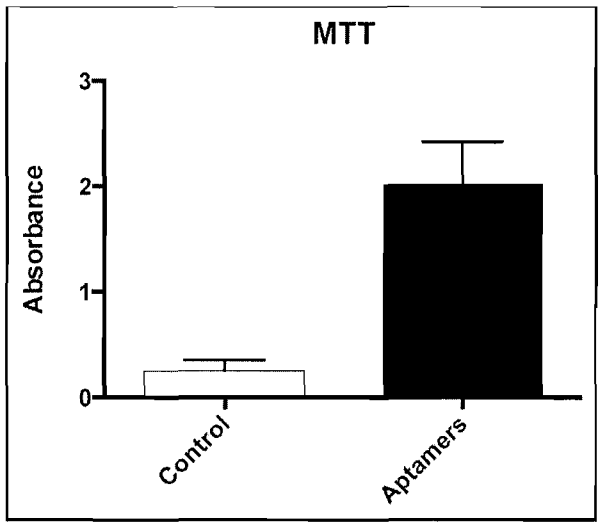

fig. 10

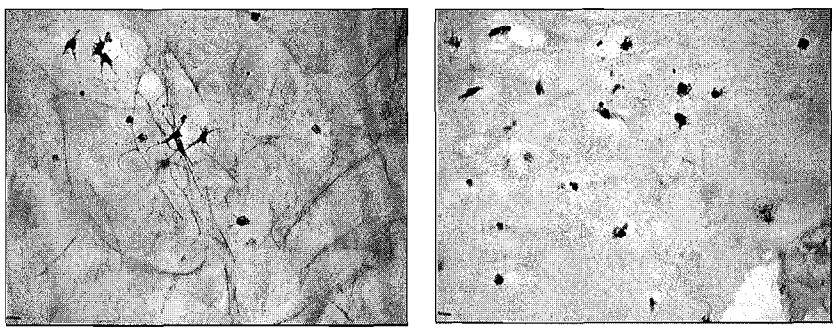

fig. 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/053502

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L27/34 A61K31/7088 C12N15/115 C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61L A61K C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/148493 A1 (BALLERSTADT RALPH [US] ET AL) 11 June 2009 (2009-06-11) page 1, paragraph 6-9 page 2, paragraph 11-17 page 4, paragraphs 41-43,45 -----	1-19
X	US 2011/150964 A1 (BORCK ALEXANDER [DE]) 23 June 2011 (2011-06-23) page 1, paragraph 10 - page 2, paragraph 24 page 2, paragraph 26-32 page 3, paragraphs 39,40 page 7, paragraphs 99,103 ----- -/--	1-19

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 March 2014	04/04/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bonello, Steve

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JAN HOFFMANN ET AL: "Immobilized DNA aptamers used as potent attractors for porcine endothelial precursor cells", JOURNAL OF BIOMEDICAL MATERIALS RESEARCH PART A, vol. 84A, no. 3, 16 July 2007 (2007-07-16) , pages 614-621, XP055075888, ISSN: 1549-3296, DOI: 10.1002/jbm.a.31309 page 615, column 1, paragraphs 3,4 page 619, column 2, paragraph 4 - page 620, column 1, paragraph 3 -----	1-3,5-18

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/053502

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2009148493 A1	11-06-2009	NONE	
US 2011150964 A1	23-06-2011	EP 2338537 A2	29-06-2011
		US 2011150964 A1	23-06-2011