

COMBINATION COMPRISING AN AGENT PROVIDING A SIGNAL, AN
IMPLANT MATERIAL AND A DRUG

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prior art to the present invention. It is noted that in this disclosure and particularly in
the claims and/or paragraphs, terms such as “comprises”, “comprised”, “comprising”
15 and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can
mean “includes”, “included”, “including”, and the like; and that terms such as
“consisting essentially of” and “consists essentially of” have the meaning ascribed to
them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but
exclude elements that are found in the prior art or that affect a basic or novel
20 characteristic of the invention. The embodiments of the present invention are
disclosed herein or are obvious from and encompassed by, the detailed description.
The detailed description, given by way of example, but not intended to limit the
invention solely to the specific embodiments described, may best be understood in
conjunction with the accompanying drawings.

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The present invention relates to compositions or combinations of materials for non-
degradable and degradable implantable medical devices with regard to the setup of
their signal generating properties and control of their therapeutic effectiveness, as
well as to a method for the control of degradation of degradable or partially
30 degradable medical devices composed like this, based on their signal generation, and

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to a method for supervision of their therapeutic effectiveness and/or the release of therapeutically active ingredients from such devices.

- Ultra-short term implants, short term implants such as orthopedic-surgical screws, plates, nails or catheters and injection needles, as well as long term implants like joint prostheses, artificial heart valves, vascular prostheses, stents, also subcutaneous or intramuscular types of implant are manufactured from different types of materials, which are selected according to their specific biochemical and mechanical properties. These materials must be suitable for permanent use in the body, not release toxic materials and have specific mechanical and biochemical properties. The manufacture of such implants with new materials is increasingly allowing the functionality of the implants to be improved. In particular in this respect, systems are used which are partially degradable/dissolvable or completely (bio-)degradable.
- A significant problem of such implants is that with the use of new materials limited physical properties are provided, as in medical imaging methods, for example during the application, follow-up or control of the correct anatomical position or for other diagnostic or therapeutic reasons, for example, inadequate radiopaque or diamagnetic, paramagnetic, super paramagnetic or ferromagnetic properties. In particular, biodegradable materials such as polylactonic acid and its derivatives, collagens, albumin, gelatin, hyaluronic acid, starch, cellulose and the like are typically radiolucent. This also applies for example to polymers like polyurethanes, poly(ethylene vinyl acetate), silicones, acrylic polymers like polyacrylic acids, polymethylacrylic acid, polyacrylcianoacrylate; polyethylene, polypropylene, polyamide, poly(ester urethane), poly(ether urethane), poly(ester urea), polyethers like polyethylene oxide, polypropylene oxide, pluronics, polytetramethylene glycol; vinyl polymers like polyvinylpyrrolidone, poly(vinyl alcohol), poly(vinylacetatephthalate); parylenes, which based on material properties are

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excellently suited for biomedical applications. In particular these are also suited for non-resorbable medical implants, which consist of polymers or composite materials and are primarily only weak radiopaques or are radiolucent.

- 5 In contrast to this there are other requirements of materials, which are exposed to diagnostics by means of magnetic resonance tomography methods. In contrast to conventional X-ray diagnostics, which is based on the application of ionizing radiation, magnetic resonance tomography (MRI) is not based on ionizing radiation but instead on the production of a magnetic field, radio-frequency energy and
- 10 magnetic field gradients. The signals produced are based predominantly on the measured relaxation times T1 (longitudinal) and T2 (transversal) of excited protons and the proton density in the tissues. So, typically, contrast materials are applied, in order, for example to influence the proton densities and/or relaxation times produced in tissues or tissue sections, for example the T1, T2 or proton densities.

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- Another problem is that implantable medical devices are typically modified, in order to improve their imaging properties. For example, radiopaque fillers are added to polymeric materials in order to improve their visibility. In connection with this, typical fillers are BaSO₄, bismuth sub carbonate or metals like tungsten, or other
- 20 bismuth salts like bismuth sub nitrate and bismuth oxide [see U.S. Patent 3,618,614]. Other types of modifications are for example the incorporation of halogenated compounds or groups into the polymer matrix. As examples of this, U.S. Patents 4,722,344, 5,177,170 and 5,346,981 are cited here.

- 25 Disadvantages of such fillers are, for example, that fundamental material properties are altered, such as the optical properties, mechanical strength, flexibility, acid and alkali resistance. Another disadvantage of the methods described is that for example a minimum amount of radiopaque fillers or halogenated components must generally

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be added in order to produce any significant radiopaque properties, however the solubility of such filler materials in the polymer precursors is limited.

Comparable problems definitely exist throughout, in the case for example of metal-based implant materials, intravascular devices, which are in the body temporarily or permanently. Exemplary of such devices, stents should be cited, which typically are made of metals. The application of stents is a necessarily invasive method wherein it is of significant clinical importance that the stent be positioned correctly. For this, visualization by means of an image forming method, for example an X-ray based method both during and after the application, is customary. Based on the alloys used and the low material weights, with thin walls or low material strengths, the visibility is only weak when it exists at all. Certain radiopaque components which absorb ionizing radiation, also those metal alloys which are biocompatible, can be employed according to the prior art, however these typically have a negative impact on the mechanical and (bio-)chemical properties.

Other prior art methods are based on the application of band markers, which are pressed on, glued on or electrochemically deposited radiopaque materials or metallic coatings.

The disadvantages of such solutions are for example that the band marker may be dislodged or detaches completely during the application, further that they damage the tissue of the inner wall of the vessel mechanically and traumatize the surrounding tissue, if they are sharp-edged or are attached at the outer edges of the implant. In the worst case band markers cause complications which can make the implant useless. Moreover such bands can lead to rough surfaces which can lead to development of thromboses later on.

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Other prior art methods utilize metal-based coatings, which can be produced by CVD, PVD or electrochemical methods. However in order to be able to obtain sufficient radiopaque coatings, coating thicknesses to produce adhesion onto the metallic substrates are not sufficient to satisfy the mechanical demands put on such implants, which are insufficient to ensure the safety and effectiveness of such an implant.

On the other hand, electrochemical methods are of only limited suitability, since the deposition of coatings is typically associated with rough surfaces with worsening of haemo-compatibility, or even, depending on the underlying substrate, to embrittlement, corrosion or lead to other impairment of the underlying material properties of the substrate. Such limitations are known typically for titanium based alloys, whose mechanical properties deteriorate significantly as a result of embrittlement and therewith the functionality of the implant.

Ion beam assisted implantations of radiopaque materials have the disadvantage that they are extremely expensive, cost intensive and are of only limited applicability, especially since the evaporation out from the molten metal takes place, in an amount that exceeds several times the actual amount to be put on, the deposition and the growth of the coating becomes irregular and difficult to control and for example makes the implantation of alloys from melts difficult to carry out in a controlled manner due to the differing evaporation rates of the elements.

Further known are implantable medical devices which contain active ingredients in the implant body or in parts of the implant body or in coatings. Through complete or partial degradation or without degradation of the implant body, of parts of the implant body or of coatings, the active ingredients are released. Such implantable medical devices are known to those skilled in the art under the designation

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"combinatorial devices". It is particularly desired, for non-degradable and degradable materials which contain active ingredients, to control the release of the active ingredients *in vivo*.

5 A review of the prior art shows that such devices combined with active ingredients do not allow for an effective control of active ingredient release from outside the body, since the active ingredients used do not themselves have at their disposal any signal generating properties. Also, if the materials used in which the active ingredients are embedded degradable or dissolvable in the presence of physiologic
10 fluids, their degradation rate does not correlate with the release of active ingredients, even if the matrix materials are visible by signal detecting methods. An example of this, especially considering the prior art, is represented by drug-eluting stents, whose release of active ingredients is determined on the basis of costly *in-vitro* and *in-vivo* studies in very expensive pre-clinical studies. However, in such clinical studies
15 information on clinical usefulness of the devices can be gathered only by means of indirect parameters such as restenosis rates, wall thickening of the concerned vessel, ability to penetrate etc. *ex post*, months after implantation. For the actual limitations in regard to control of active ingredient release, reference is made on this point to Schwart et al., Circulation. 2002; 106:1867.

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There is therefore a need for medical implants which are detectable for diagnostic and therapeutic purposes during or after their application by image generating methods, which are based on ionizing radiation, radio frequency radiation, fluorescence or luminescence, or sound based methods and the like.

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In particular there is a need for visible implants in image producing methods which are completely or partially biodegradable or bio-erodible, and the rate of degradation in corresponding non-invasive measurement and detection methods, for example

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image producing method, is controllable over the residence time and permits a correlation between implant effectiveness and therapeutic result over the acquisition of implantation/tissue limits and new tissue growth.

5 **Summary of the invention**

Therefore, the present invention in one aspect provides implants being made visible in image producing methods, which preferably can be made visible at the same time in as many as possible image producing methods based on different physical principles. The embodiments of the present invention allow for a control of the correct anatomical positioning of an implantable medical device in situ during the application, conventionally by means of radiographic methods, but also for the follow-up and monitoring of the therapeutic effectiveness, with the use of non-stressing or non-invasive based detection methods for example based on MRI.

Furthermore, the invention provides assemblies of implantable medical devices, which contain therapeutically active ingredients and release them controllably, for example, in that for degradable or partially degradable components the extent of the degradation is correlatable with the extent of release of therapeutically active ingredients, or, in that for non-degradable, active ingredient releasing implantable devices the active ingredient is coupled to a signal producing agent and the depletion of signals in the device or parts of the device indicate the extent of the release of the active ingredients.

Also, in further aspects the present invention allows to control the release of active ingredients from an implant, in order to locally detect the enrichment of active ingredients, which are released from an implantable medical device, into specific compartments of the organism, organs, tissues or cells, especially in specific cell types. Additionally, the present invention provides methods for and implantable

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medical devices whose therapeutic effectiveness is controllable with or without active ingredient release by means of the enrichment of signal producing agents in compartments of the organism, organs, tissues or cells, especially in specific cell types, wherein these already have inherent signal generating properties, or are only
5 transformed *in vivo* into signal generating agents by biological mechanisms. This can then be especially preferred, if for example an implantable medical device is applied as tissue substitute in malign tissue, and it changes after metastasis or tumor removal, and fulfills the purpose, the release of signal generating agents, recurrence in immediate or communicable surroundings of the implant by means of selective
10 enrichment, brought about for example through targeting groups, to make them visible in such altered cell or tissue types.

Also, the present invention provides methods which make it possible not to impair the material composition of the implant through mixing in of detectable substances
15 which in this way limit or even destroy the functionality.

In one aspect, the present invention makes available a composition or combination for implantable medical devices or components of implantable medical devices, which is adjustable with regard to the signal generating properties of it.

20 In a further aspect, the invention makes available a composition or combination for implantable medical devices which can be adjusted with regard to the period of identification, i.e. the temporal availability of detectable properties.

In a still further aspect, the invention makes available a composition or combination
25 for implantable medical devices which is detectable by different measurement and detection methods.

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Additionally, it is an aspect of the invention to make available a composition or combination for implantable medical devices which allows to detect the range of the release of therapeutically active ingredients by means of signal generating methods, especially the release of therapeutically active ingredients from implantable medical
5 devices, or from components of implantable medical devices or the enrichment of active ingredients which are released from implantable medical devices or from components of implantable medical devices in compartments of the organism, organs, tissues or tissue or cell types, or both.

10 A further aspect of the invention makes available a composition or combination for implantable medical devices which allows to control the implant effectiveness, preferably through measurement and detection methods which make the implant-tissue boundaries visible, or by release of signal generating agents and/or their enrichment in compartments of the organism, organ, tissues or tissue or cell types,
15 preferably in the immediate vicinity of the implanted medical device.

A still further aspect of the invention makes available a composition or combination for implantable medical devices which releases signal generating agents for diagnostic and/or therapeutic purposes after insertion into an animal or human body.

20 In a preferred embodiment of this aspect, the signal-generating and therapeutic/diagnostic agents are released substantially simultaneously, and most preferably the agents are coupled or bonded to each other.

In a further aspect, the invention makes available a composition or combination for
25 implantable medical devices or components of implantable medical devices, which allows accomplishment of setting up of signal generating properties, i.e. to adjust herewith in which measurement and detection methods the device or its components are detectable, or to set up whether the release of signal generating agents and/or

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therapeutically active ingredients results directly from the release from the implantable medical device or components of the implantable medical devices, i.e. resulting in a depletion of the signal generating agents in the device or the components of the device, or follows indirectly, thus through enrichment in
5 compartments of the organism, of organs, tissues or tissue or cell types or both.

In a still further aspect, the present invention provides a method for the control of degradation of degradable or partially degradable medical devices composed like this, based on their signal generation, and a method for supervision of their
10 therapeutic effectiveness and/or the release of therapeutically active ingredients from such devices.

In a further aspect, the invention makes available a method, which makes possible the determination of the extent of release of active ingredients from an implantable
15 medical device or a component of an implantable medical device, and to make available a method which makes possible determination of the extent of the active ingredient enrichment of active ingredients which are released from an implantable medical device or a component of an implantable medical device.

20 According to one embodiment, the invention provides the following combination, comprising:

- a. at least one signal generating agent, which leads directly or indirectly to detectable signals in a physical, chemical and/or biological
25 measurement or detection method,
- b. at least one material for the preparation of an implantable medical device and/or at least one component of an implantable medical device,

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- 5 c. at least one therapeutically active ingredient, which either directly or indirectly fulfills a therapeutic function in an animal or human organism and is directly or indirectly released in an animal or human organism from an implantable medical device or a component of the implantable medical device.

10 In another embodiment, the invention provides an implantable medical device or component thereof, comprising at least one signal-generating agent and at least one therapeutically active agent as defined hereinbelow. In a preferred embodiment, the signal-generating agents and therapeutic agents can be released substantially simultaneously from the device, after its insertion into the human or animal body.

15 In a further embodiment, the present invention includes a combination for the manufacture of implantable medical devices, comprising a first and a second signal-generating agent, which lead directly or indirectly to detectable signals in a physical, chemical and/or biological measurement or verification method, wherein the first agent in a method, in which the second agent leads to detectable signals is essentially not detectable.

20 Preferably, such combinations as mentioned above can be used in the manufacture of implantable medical devices for insertion into the human or animal body, for drug-delivery implants and the like, for example as a coating or a component of a coating of the device, or as at least a part or the construction material of the device itself.

25 In a further embodiment, the present invention is directed to a method for the determination of the extent of the release of an active agent from a completely or partially degradable or dissolvable implantable medical device, or component thereof, the device comprising at least one signal-generating agent, which leads directly or indirectly to detectable signals in a physical, chemical and/or biological

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measurement or verification method, especially in an imaging method, and at least one therapeutically active agent to be released in a human or animal organism, and wherein the device at least partially releases the therapeutically active agent(s) together with the signal generating agent(s) in the presence of physiologic fluids, for example after insertion of the device into a human or animal body, and wherein the extent of active agent release can be determined by detecting the released signal-generating agent with the use of non-invasive imaging methods.

In a still further embodiment, the present invention is directed to a method for the determination of the extent of the release of an active agent from a non-degradable implantable medical device or a component thereof, manufactured by use of a combination, which comprises a signal-generating agent, which leads directly or indirectly to detectable signals in a physical, chemical and/or biological measurement or verification method, especially in an imaging method, as well as a therapeutically active agent to be released in a human or animal organism, and wherein the extent of active agent release can be determined by detecting the released signal-generating agent with the use of non-invasive imaging methods.

Preferably, microspheres, optionally comprising metals and or drugs, intended for direct injection or incorporation into the human or animal body are excluded from the embodiments of the present invention.

SIGNAL GENERATING MATERIAL

In accordance with the invention the signal generating material can be selected from inorganic, organic or inorganic-organic composites which are degradable, partially degradable or non-degradable. Under signal generating materials are to be understood those which in physical, chemical and/or biological measurement and verification methods lead to detectable signals, for example in image-producing

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methods. It is unimportant for the present invention, whether the signal processing is carried out exclusively for diagnostic or therapeutic purposes.

Typical imaging methods are for example radiographic methods, which are based on ionizing radiation, for example conventional X-ray methods and X-ray based split image methods such as computer tomography, neutron transmission tomography, radiofrequency magnetization such as magnetic resonance tomography, further by radionuclide-based methods such as scintigraphy, Single Photon Emission Computed Tomography (SPECT), Positron Emission Computed Tomography (PET),
ultrasound-based methods or fluoroscopic methods or luminescence or fluorescence based methods such as Intravasal Fluorescence Spectroscopy, Raman spectroscopy, Fluorescence Emission Spectroscopy, Electrical Impedance Spectroscopy, colorimetry, optical coherence tomography, etc, further Electron Spin Resonance (ESR), Radio Frequency (RF) and Microwave Laser and similar methods.

Signal generating agents can be metal-based from the group of metals, metal oxides, metal carbides, metal nitrides, metal oxynitrides, metal carbonitrides, metal oxycarbides, metal oxynitrides, metal oxycarbonitrides, metal hydrides, metal alkoxides, metal halides, inorganic or organic metal salts, metal polymers, metallocenes, and other organometallic compounds, chosen from powders, solutions, dispersions, suspensions, emulsions.

Preferred metal based agents are especially nanomorphous nanoparticles from 0-valent metals, metal oxides or mixtures there from. The metals or metal oxides used can also be magnetic; examples are – without excluding other metals – iron, cobalt, nickel, manganese or mixtures thereof, for example iron-platinum mixtures, or as an example for magnetic metal oxides, iron oxide and ferrites.

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It can be preferred to use semi conducting nanoparticles, examples for this are semiconductors from group II-VI, group III-V, group IV. Group II-VI - semiconductors are for example MgS, MgSe, MgTe, CaS, CaSe, CaTe, SrS, SrSe, SrTe, BaS, BaSe, BaTe, ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe or
5 mixtures thereof. Examples of group III-V semiconductors are for example GaAs, GaN, GaP, GaSb, InGaAs, InP, InN, InSb, InAs, AlAs, AlP, AlSb, AlS, and mixtures thereof are preferred. Germanium, lead and silicon are selected as exemplary of group IV semiconductors. The semiconductors can moreover also contain mixtures of semiconductors from more than one group, all groups mentioned above are
10 included.

It can moreover be preferred to choose complex formed metal-based nanoparticles. Included here are so-called Core-Shell configurations, as described explicitly by Peng et al., "Epitaxial Growth of Highly Luminescent CdSe/CdS Core/Shell
15 Nanoparticles with Photo stability and Electronic Accessibility", Journal of the American Chemical Society, (1997) 119:7019-7029, and included herewith explicitly per reference. Preferred here are semi conducting nanoparticles, which form a core with a diameter of 1-30 nm, especially preferred of 1-15 nm, onto which other semi conducting nanoparticles crystallize in 1- 50 monolayers, especially preferred are 1-
20 15 monolayers. In this case core and shell can be present in any desired combinations as described above, in special embodiments CdSe and CdTe are preferred as the core and CdS and ZnS as the shell .

In a special embodiment the signal producing nanoparticles have absorption
25 properties for radiation in the wavelength regions of gamma rays up to microwave radiation, or have the property of emitting radiation, especially in the range of 60 nm or less, wherein through corresponding selection of the particle size and diameter of the core and shell it can be preferred, to set the emission of light quanta in ranges

from 20 to 1000 nm or to select mixtures of such particles which emit quanta of different wavelengths if exposed to radiation themselves. In a preferred embodiment the selected nanoparticles are fluorescent, especially without quenching.

- 5 Further, signal producing metal-based agents can be selected from salts or metal ions, which preferably have paramagnetic properties, for example lead (II), bismuth (II), bismuth (III), chromium (III), manganese (II), manganese (III), iron (II), iron (III), cobalt (II), nickel (II), copper (II), praseodymium (III), neodymium (III), samarium (III), or ytterbium (III), holmium (III) or erbium (III) and the like. Based
10 on especially pronounced magnetic moments, especially gadolinium (III), terbium (III), dysprosium (III), holmium (III) and erbium (III) are mostly preferred. Further one can select from radioisotopes. Examples of a few applicable radioisotopes include H 3, Be 10, O 15, Ca 49, Fe 60, In 111, Pb 210, Ra 220, Ra 224 and the like. Typically such ions are present as chelates or complexes, wherein for example as
15 chelating agents or ligands for lanthanides and paramagnetic ions compounds such as diethylenetriamine pentaacetic acid ("DTPA"), ethylenediamine tetra acetic acid ("EDTA"), or tetraazacyclododecane-N,N', N'',N'''-tetra acetic acid ("DOTA") are used. Other typical organic complexing agents are for example published in Alexander, Chem. Rev. 95:273-342 (1995) and Jackels, Pharm. Med. Imag, Section
20 III, Chap. 20, p645 (1990). Other usable chelating agents in the present invention are found in U.S. Patents 5,155,215, 5,087,440, 5,219,553, 5,188,816, 4,885,363, 5,358,704, 5,262,532, and Meyer et al., Invest. Radiol. 25: S53 (1990), further U.S. Patents 5,188,816, 5,358,704, 4,885,363, and 5,219,553. Preferred mostly are salts and chelates from the lanthanide group with the atomic numbers 57-83 or the
25 transition metals with the atomic numbers 21-29, or 42 or 44.

Especially preferred are paramagnetic perfluoroalkyl containing compounds which for example are described in German laid-open patents DE 196 03 033, DE 197 29

013 and in WO 97/26017, which in accordance with the invention are incorporated hereto, by reference further diamagnetic perfluoroalkyl containing substances of the general formula:

R<PF>-L<II>-G<III>, wherein R<PF> represents a perfluoroalkyl group with 4 to 30 carbon atoms, L<II> stands for a linker and G<III> for a hydrophilic group. The linker L is a direct bond, an -SO₂- group or a straight or branched carbon chain with up to 20 carbon atoms which can be substituted with one or more -OH, -COO<->, -SO₃-groups and/or if necessary one or more -O-, -S-, -CO-, -CONH-, -NHCO-, -CONR-, -NRCO-, -SO₂-, -PO₄-, -NH-, -NR-groups, an aryl ring or contain a piperazine, wherein R stands for a C₁ to C₂₀ alkyl group, which again can contain and/or have one or a plurality of O atoms and/or be substituted with -COO<-> or SO₃- groups.

The hydrophilic group G<III> can be selected from a mono or disaccharide, one or a plurality of -COO<-> or -SO₃<->-groups, a dicarboxylic acid, an isophthalic acid, a picolinic acid, a benzenesulfonic acid, a tetrahydropyranedicarboxylic acid, a 2,6-pyridinedicarboxylic acid, a quaternary ammonium ion, an aminopolycarboxylic acid, an aminodipolyethyleneglycol sulfonic acid, an aminopolyethyleneglycol group, an SO₂-(CH₂)₂-OH-group, a polyhydroxyalkyl chain with at least two hydroxyl groups or one or a plurality of polyethylene glycol chains having at least two glycol units, wherein the polyethylene glycol chains are terminated by an -OH or -OCH₃- group, or similar linkages. In this connection the published German patent DE 199 48 651 explicitly incorporated into the invention by reference.

It can be preferred in special embodiments to choose paramagnetic metals in the form of metal complexes with phthalocyanines, especially as described in Phthalocyanine Properties and Applications, Vol. 14, C. C. Leznoff and A. B. P. Lever, VCH Ed., wherein as examples to mention are octa(1,4,7,10-

tetraoxaundecyl)Gd-phthalocyanine, octa(1,4,7,10-tetraoxaundecyl)Gd-phthalocyanine, octa(1,4,7,10-tetraoxaundecyl)Mn-phthalocyanine, octa(1,4,7,10-tetraoxaundecyl)Mn-phthalocyanine, as described in U.S. 2004214810 and herewith explicitly by reference.

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It can further be preferred to select from super-paramagnetic, ferromagnetic or ferrimagnetic signal generating agents. For example among magnetic metals, alloys are preferred, among ferrites like gamma iron oxide, magnetites or cobalt-, nickel- or manganese-ferrites, corresponding agents are preferably selected, especially particles as described in WO83/03920, WO83/01738, WO85/02772 and WO89/03675, in U.S. Pat. 4,452,773, U.S. Pat. 4,675,173, in WO88/00060 as well as U.S. Pat. 4,770,183, in WO90/01295 and in W090/01899, which are explicitly incorporated by reference, and others.

15 Further, magnetic, paramagnetic, diamagnetic or super paramagnetic metal oxide crystals having diameters of less than 4000 Angstroms are especially preferred as degradable non-organic agents. Suitable metal oxides can be selected from iron oxide, cobalt oxides, iridium oxides or the like, which provide suitable signal producing properties and which have especially biocompatible properties or are
20 biodegradable. Mostly preferred are crystalline agents of this group having diameters smaller than 500 Angstroms. These crystals can be associated covalently or non-covalently with macromolecular species and are modified like the metal-based signal generating agents described above.

25 Further, zeolite containing paramagnets and gadolinium containing nanoparticles are selected from polyoxometallates, preferably of the lanthanides, (e.g., K9GdW10O36).

- It is preferred to limit the average particle size of the magnetic signal producing agents to maximal 5 μm in order to optimize the image producing properties, and it is especially preferred that the magnetic signal producing particles be of a size from 2 nm up to 1 μm , most preferably 5 nm to 200 nm. The super paramagnetic signal
- 5 producing agents can be chosen for example from the group of so-called SPIOs (super paramagnetic iron oxides) with a particle size larger than 50 nm or from the group of the USPIOs (ultra small super paramagnetic iron oxides) with particle sizes smaller than 50 nm.
- 10 In accordance with the invention it can be preferred to select signal generating agents from the group of endohedral fullerenes, as disclosed for example in U.S. Patent 5,688,486 or WO 9315768, which are incorporated by reference. It is further preferred to select fullerene derivatives and their metal complexes. Especially preferred are fullerene species, which comprise carbon clusters having 60, 70, 76, 78,
- 15 82, 84, 90, 96 or more carbon atoms. An overview of such species can be gathered from European patent 1331226A2 and is explicitly incorporated herein by reference. Further metal fullerenes or endohedral carbon-carbon nanoparticles with arbitrary metal-based components can also be selected. Such endohedral fullerenes or endometallo fullerenes are particularly preferred, which for example contain rare
- 20 earths such as cerium, neodymium, samarium, europium, gadolinium, terbium, dysprosium or holmium. Moreover it can be especially preferred to use carbon coated metallic nanoparticles such as carbides. The choice of nanomorphous carbon species is not limited to fullerenes, since it can be preferred to select from other nanomorphous carbon species such as nanotubes, onions, etc. In another embodiment
- 25 it can be preferred to select fullerene species from non-endohedral or endohedral forms, which contain halogenated, preferably iodated, groups, as disclosed in U.S. Patent 6,660,248, which is incorporated herewith by reference.

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In certain embodiments mixtures of such signal generating agents of different specifications are also used, depending on the desired properties of the wanted signal generating material properties. The signal producing agents used generally can have a size of 0.5 nm to 1000 nm, preferably 0.5 nm to 900 nm, especially preferred from 5 0.7 to 100 nm. In this connection the metal-based nanoparticles can be provided as a powder, in polar, non-polar or amphiphilic solutions, dispersions, suspensions or emulsions. Nanoparticles are easily modifiable based on their large surface to volume ratios. The nanoparticles to be selected can for example be modified non-covalently by means of hydrophobic ligands, for example with trioctylphosphine, or 10 be covalently modified. Examples of covalent ligands are thiol fatty acids, amino fatty acids, fatty acid alcohols, fatty acids, fatty acid ester groups or mixtures thereof, for example oleic acid and oleylamine.

In accordance with the invention the signal producing agents can be encapsulated in 15 micelles or liposomes with the use of amphiphilic components, or may be encapsulated in polymeric shells, wherein the micelles/liposomes can have a diameter of 2 nm to 800 nm, preferably from 5 to 200 nm, especially preferred from 10 to 25 nm. The size of the micelles/liposomes is, without committing to a specific theory, dependant on the number of hydrophobic and hydrophilic groups, the 20 molecular weight of the nanoparticles and the aggregation number. In aqueous solutions the use of branched or unbranched amphiphilic substances, is especially preferred in order to achieve the encapsulation of signal generating agents in liposomes/micelles. The hydrophobic nucleus of the micelles hereby contains in a preferred embodiment a multiplicity of hydrophobic groups, preferably between 1 25 and 200, especially preferred between 1 and 100 and mostly preferred between 1 and 30 according to the desired setting of the micelle size.

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Hydrophobic groups consist preferably of hydrocarbon groups or residues or silicon-containing residues, for example polysiloxane chains. Furthermore, they can preferably be selected from hydrocarbon-based monomers, oligomers and polymers, or from lipids or phospholipids or comprise combinations hereof, especially glyceryl

5 esters such as phosphatidyl ethanolamine, phosphatidyl choline, or polyglycolides, polylactides, polymethacrylate, polyvinylbutylether, polystyrene, polycyclopentadienylmethylnorbornene, polyethylenepropylene, polyethylene, polyisobutylene, polysiloxane. Further for encapsulation in micelles hydrophilic polymers are also selected, especially preferred polystyrenesulfonic acid, poly-N-

10 alkylvinylpyridiniumhalides, poly(meth)acrylic acid, polyamino acids, poly-N-vinylpyrrolidone, polyhydroxyethylmethacrylate, polyvinyl ether, polyethylene glycol, polypropylene oxide, polysaccharides like agarose, dextrane, starches, cellulose, amylose, amylopectin, or polyethylene glycol or polyethylene imine of any desired molecular weight, depending on the desired micelles property. Further,

15 mixtures of hydrophobic or hydrophilic polymers can be used or such lipid-polymer compositions employed. In a further special embodiment, the polymers are used as conjugated block polymers, wherein hydrophobic and also hydrophilic polymers or any desired mixtures there of can be selected as 2-, 3- or multi-block copolymers.

20 Such signal generating agents encapsulated in micelles can moreover be functionalized, while linker (groups) are attached at any desired position, preferably amino-, thiol, carboxyl-, hydroxyl-, succinimidyl, maleimidyl, biotin, aldehyde- or nitrilotriacetate groups, to which any desired corresponding chemically covalent or non-covalent other molecules or compositions can be bound according to the prior

25 art. Here, especially biological molecules such as proteins, peptides, amino acids, polypeptides, lipoproteins, glycosaminoglycans, DNA, RNA or similar bio molecules are preferred especially.

It is moreover preferred to select signal generating agents from non-metal-based signal generating agents, for example from the group of X-ray contrast agents, which can be ionic or non-ionic. Among the ionic contrast agents are included salts of 3-acetyl amino-2,4,6-triiodobenzoic acid, 3,5-diacetamido-2,4,6-triiodobenzoic acid, 2,4,6-triiodo-3,5-dipropionamido-benzoic acid, 3-acetyl amino-5-((acetyl amino)methyl)-2,4,6-triiodobenzoic acid, 3-acetyl amino-5-(acetyl methyl amino)-2,4,6-triiodobenzoic acid, 5-acetamido-2,4,6-triiodo-N-((methylcarbamoyl)methyl)-isophthalamic acid, 5-(2-methoxyacetamido)-2,4,6-triiodo-N-[2-hydroxy-1-(methylcarbamoyl)-ethoxy 1]-isophthalamic acid, 5-acetamido-2,4,6-triiodo-N-methylisophthalamic acid, 5-acetamido-2,4,6-triiodo-N-(2-hydroxyethyl)-isophthalamic acid 2-[[2,4,6-triiodo-3[(1-oxobutyl)-amino]phenyl]methyl]-butanoic acid, beta-(3-amino-2,4,6-triiodophenyl)-alpha-ethyl-propanoic acid, 3-ethyl-3-hydroxy-2,4,6-triiodophenyl-propanoic acid, 3-[[[(dimethylamino)-methyl]amino]-2,4,6-triiodophenyl-propanoic acid (see Chem. Ber. 93: 2347 (1960)), alpha-ethyl-(2,4,6-triiodo-3-(2-oxo-1-pyrrolidinyl)-phenyl)-propanoic acid, 2-[2-[3-(acetyl amino)-2,4,6-triiodophenoxy]ethoxymethyl]butanoic acid, N-(3-amino-2,4,6-triiodobenzoyl)-N-phenyl-.beta.-aminopropanoic acid, 3-acetyl-[(3-amino-2,4,6-triiodophenyl)amino]-2-methylpropanoic acid, 5-[(3-amino-2,4,6-triiodophenyl)methyl amino]-5-oxypentanoic acid, 4-[ethyl-[2,4,6-triiodo-3-(methyl amino)-phenyl]amino]-4-oxo-butanoic acid, 3,3'-oxy-bis[2,1-ethanediyl-oxy-(1-oxo-2,1-ethanediyl)imino]bis-2,4,6-triiodobenzoic acid, 4,7,10,13-tetraoxahexadecane-1,16-diyl-bis(3-carboxy-2,4,6-triiodoanilide), 5,5'-(azelaoyldiimino)-bis[2,4,6-triiodo-3-(acetyl amino)methyl-benzoic acid], 5,5'-(apidolediimino)bis(2,4,6-triiodo-N-methyl-isophthalamic acid), 5,5'-(sebacoyl-diimino)-bis(2,4,6-triiodo-N-methylisophthalamic acid), 5,5'-[N,N-diacetyl-(4,9-dioxy-2,11-dihydroxy-1,12-dodecanediyl)diimino]bis(2,4,6-triiodo-N-methyl-isophthalamic acid), 5,5'-(nitrilo-triacetyltriimino)tris(2,4,6-triiodo-N-methyl-isophthalamic acid), 4-hydroxy-3,5-diiodo-alpha-phenylbenzenepropanoic acid, 3,5-diiodo-4-oxo-1(4H)-pyridine

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acetic acid, 1,4-dihydro-3,5-diiodo-1-methyl-4-oxo-2,6-pyridinedicarboxylic acid, 5-iodo-2-oxo-1(2H)-pyridine acetic acid, and N-(2-hydroxyethyl)-2,4,6-triiodo-5-[2,4,6-triiodo-3-(N-methylacetamido)-5-(methylcarbomoyl)benzamino]acetamido]-isophthalamide acid, and the like especially preferred, as well as other ionic X-ray contrast agents suggested in the literature, for example in J. Am. Pharm. Assoc., Sci. Ed. 42:721 (1953), Swiss Patent 480071, JACS 78:3210 (1956), German patent 2229360, U.S. Patent 3,476,802, Arch. Pharm. (Weinheim, Germany) 306: 11 834 (1973), J. Med. Chem. 6: 24 (1963), FR-M-6777, Pharmazie 16: 389 (1961), U.S. Patents 2,705,726, U.S. Patent 2,895,988, Chem. Ber. 93:2347(1960), SA-A-68/01614, Acta Radiol. 12: 882 (1972), British Patent 870321, Rec. Trav. Chim. 87: 308 (1968), East German Patent 67209, German Patent 2050217, German Patent 2405652, Farm Ed. Sci. 28: 912(1973), Farm Ed. Sci. 28: 996 (1973), J. Med. Chem. 9: 964 (1966), Arzheim.-Forsch 14: 451 (1964), SE-A-344166, British Patent 1346796, U.S. Patent 2,551,696, U.S. Patent 1,993,039, Ann 494: 284 (1932), J. Pharm. Soc. (Japan) 50: 727 (1930), und U.S. Patent 4,005,188. The disclosures listed here are explicitly incorporated by reference into the invention.

Examples of applicable non-ionic X-ray contrast agents in accordance with the invention are metrizamide as disclosed in DE-A-2031724, iopamidol as disclosed in BE-A-836355, iohexol as disclosed in GB-A-1548594, iotrolan as disclosed in EP-A-33426, iodecimol as disclosed in EP-A-49745, iodixanol as in EP-A-108638, iogluconol as disclosed in U.S. Patent 4,314,055, ioglucomide as disclosed in BE-A-846657, iogluconol as in DE-A-2456685, iogulamide as in BE-A-882309, iomeprol as in EP-A-26281, iopentol as EP-A-105752, iopromide as in DE-A-2909439, iosarcol as in DE-A-3407473, iosimide as in DE-A-3001292, iotasul as in EP-A-22056, iovarsul as disclosed in EP-A-83964 or ioxilan in WO87/00757, and the like. The references provided are incorporated herewith in accordance with the invention.

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In some embodiments it is especially preferred to select agents based on nanoparticle signal generating agents, which after release into tissues and cells are incorporated or are enriched in intermediate cell compartments and/or have an especially long residence time in the organism.

- 5 Such particles are selected in a special embodiment from water-insoluble agents, in another embodiment they contain a heavy element like iodine or barium, in a third PH-50 as monomer, oligomer or polymer (iodinated aroyloxy ester having the empirical formula $C_{19}H_{23}I_3N_2O_6$, and the chemical names 6-ethoxy-6-oxohexy-3, 5-bis (acetyl amino)-2,4,6-triiodobenzoate), in a fourth embodiment an ester of
- 10 diatrizoic acid, in a fifth an iodinated aroyloxy ester or in a sixth embodiment any combinations hereof. In these embodiments particle sizes are preferred, which can be incorporated by macrophages. A corresponding method for this is disclosed in WO03039601 and agents preferred to be selected are disclosed in the publications U.S. Patents 5,322,679, 5,466,440, 5,518,187, 5,580,579, und 5,718,388, gel of
- 15 which are explicitly incorporated by reference in accordance with the invention. Especially advantageous are particularly, nanoparticles which are marked with signal generating agents or such signal generating agents like PH-50, which accumulate in intercellular spaces and can make interstitial as well as extrastitial compartments visible.

20

- Signal generating agents can be selected moreover from the group of the anionic or cationic lipids, as disclosed already in U.S. Patent 6,808,720 and explicitly incorporated herewith. Especially preferred are anionic lipids like phosphatidyl acid, phosphatidyl glycerol and their fatty acid esters, or amides of phosphatidyl
- 25 ethanolamine, like anandamide and methanandamide, phosphatidyl serine, phosphatidyl inositol and their fatty acid esters, cardiolipin, phosphatidyl ethylene glycol, acid lysolipids, palmitic acid, stearic acid, arachidonic acid, oleic acid, linoleic acid, linolenic acid, myristic acid, sulfolipids and sulfatides, free fatty acids,

both saturated and unsaturated and their negatively charged derivatives, and the like. Moreover, specially halogenated, in particular fluorinated anionic lipids are preferred. The anionic lipids preferably contain cations from the alkaline earth metals beryllium ($\text{Be}^{<+2>}$), magnesium ($\text{Mg}^{<+2>}$), calcium ($\text{Ca}^{<+2>}$), strontium

5 ($\text{Sr}^{<+2>}$) und barium ($\text{Ba}^{<+2>}$), or amphoteric ions, like aluminium ($\text{Al}^{<+3>}$), gallium ($\text{Ga}^{<+3>}$), germanium ($\text{Ge}^{<+3>}$), tin ($\text{Sn}^{<+4>}$) or lead ($\text{Pb}^{<+2>}$ and $\text{Pb}^{<+4>}$), or transition metals like titanium ($\text{Ti}^{<+3>}$ and $\text{Ti}^{<+4>}$), vanadium ($\text{V}^{<+2>}$ and $\text{V}^{<+3>}$), chromium ($\text{Cr}^{<+2>}$ and $\text{Cr}^{<+3>}$), manganese ($\text{Mn}^{<+2>}$ and $\text{Mn}^{<+3>}$), iron ($\text{Fe}^{<+2>}$ and $\text{Fe}^{<+3>}$), cobalt ($\text{Co}^{<+2>}$ und $\text{Co}^{<+3>}$), nickel

10 ($\text{Ni}^{<+2>}$ and $\text{Ni}^{<+3>}$), copper ($\text{Cu}^{<+2>}$), zinc ($\text{Zn}^{<+2>}$), zirconium ($\text{Zr}^{<+4>}$), niobium ($\text{Nb}^{<+3>}$), molybdenum ($\text{Mo}^{<+2>}$ und $\text{Mo}^{<+3>}$), cadmium ($\text{Cd}^{<+2>}$), indium ($\text{In}^{<+3>}$), tungsten ($\text{W}^{<+2>}$ and $\text{W}^{<+4>}$), osmium ($\text{Os}^{<+2>}$, $\text{Os}^{<+3>}$ und $\text{Os}^{<+4>}$), iridium ($\text{Ir}^{<+2>}$, $\text{Ir}^{<+3>}$ und $\text{Ir}^{<+4>}$), mercury ($\text{Hg}^{<+2>}$) or bismuth ($\text{Bi}^{<+3>}$), and/or rare earths like lanthanides, for example lanthanum ($\text{La}^{<+3>}$) and

15 gadolinium ($\text{Gd}^{<+3>}$). Especially preferred cations are calcium ($\text{Ca}^{<+2>}$), magnesium ($\text{Mg}^{<+2>}$) and zinc ($\text{Zn}^{<+2>}$) and paramagnetic cations like manganese ($\text{Mn}^{<+2>}$) or gadolinium ($\text{Gd}^{<+3>}$).

Cationic lipids are to be chosen from phosphatidyl ethanolamine, phosphatidylcholine,

20 Glycero-3-ethylphosphatidylcholine and their fatty acid esters, di- and trimethylammoniumpropane, di- and tri-ethylammoniumpropane and their fatty acid esters. Especially preferred derivatives are N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride ("DOTMA"); furthermore synthetic cationic lipids based on for example naturally occurring lipids like dimethyldioctadecylammonium

25 bromide, sphingolipids, sphingomyelin, lysolipids, glycolipids such as for example gangliosides GM1, sulfatides, glycosphingolipids, cholesterol und cholesterol esters or salts, N-succinyldioleoylphosphatidyl ethanolamine, 1,2,-dioleoyl-sn- glycerol, 1,3-dipalmitoyl-2-succinylglycerol, 1,2-dipalmitoyl-sn-3-succinylglycerol, 1-

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hexadecyl-2-palmitoylglycerophosphatidyl ethanolamine and palmitoyl-homocystein, mostly preferred are fluorinated, derivatized cationic lipids. Such compounds have been disclosed especially in U.S. 08/391,938 and are incorporated herewith per reference.

5

Such lipids are furthermore suitable as components of signal generating liposomes, which especially can have pH- sensitive properties as disclosed in U.S. 2004197392 and incorporated herein explicitly.

- 10 In accordance with the invention, signal generating agents can also be selected from the group of the so-called microbubbles or microballoons, which contain stable dispersions or suspensions in a liquid carrier substance. Gases to be chosen are preferably air, nitrogen, carbon dioxide, hydrogen or noble gases like helium, argon, xenon or krypton, or sulfur-containing fluorinated gases like sulfurhexafluoride,
- 15 disulfurdecafluoride or trifluoromethylsulfurpentafluoride, or for example selenium hexafluoride, or halogenated silanes like methylsilane or dimethylsilane, further short chain hydrocarbons like alkanes, specifically methane, ethane, propane, butane or pentane, or cycloalkanes like cyclopropane, cyclobutane or cyclopentane, also alkenes like ethylene, propene, propadiene or butene, or also alkynes like acetylene
- 20 or propyne. Further ethers such as dimethylether can be considered or be chosen, or ketones, or esters or halogenated short-chain hydrocarbons or any desired mixtures of the above. Especially preferred are halogenated or fluorinated hydrocarbon gases such as bromochlorodifluoromethane, chlorodifluoromethane, dichlorodifluoromethane, bromotrifluoromethane, chlorotrifluoromethane,
- 25 chloropentafluoroethane, dichlorotetrafluoroethane, chlorotrifluoroethylene, fluoroethylene, ethyl fluoride, 1,1-difluoroethane or perfluorohydrocarbons like for example perfluoroalkanes, perfluorocycloalkanes, perfluoroalkenes or perfluorinated alkynes. Especially preferred are emulsions of liquid dodecafluoropentane or

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decafluorobutane and sorbitol, or similar, as disclosed in WO-A-93/05819 and explicitly incorporated herewith by reference.

Preferably such microbubbles are selected, which are encapsulated in compounds
5 having the structure

$$R^1-X-Z;$$

$$R^2-X-Z; \text{ or}$$

$$R^3-X-Z'$$

wherein R^1 , R^2 comprises and R^3 hydrophobic groups selected from straight chain
10 alkylenes, alkyl ethers, alkyl thioethers, alkyl disulfides, polyfluoroalkylenes and
polyfluoroalkylethers, Z comprises a polar group from $CO_2-M<+>$, $SO_3<-> M<+>$,
 $SO_4<-> M<+>$, $PO_3<-> M<+>$, $PO_4<-> M<+>$, 2, $N(R)_4<+>$ or a pyridine or
substituted pyridine, and a zwitterionic group, and finally X represents a linker
which binds the polar group with the residues.

15

Gas-filled or in situ out-gassing micro spheres having a size of $< 1000 \mu m$ can be
further selected from biocompatible synthetic polymers or copolymers which
comprise monomers, dimers or oligomers or other pre-polymer to pre-stages of the
following polymerizable substances: acrylic acid, methacrylic acid, ethyleneimine,
20 crotonic acid, acryl amide, ethyl acrylate, methylmethacrylate, 2-
hydroxyethylmethacrylate (HEMA), lactonic acid, glycolic acid,
[epsilon]caprolactone, acrolein, cyanoacrylate, bisphenol A, epichlorhydrin,
hydroxyalkylacrylate, siloxane, dimethylsiloxane, ethylene oxide, ethylene glycol,
hydroxyalkylmethacrylate, N-substituted acryl amide, N-substituted
25 methacrylamides, N-vinyl-2-pyrrolidone, 2,4-pentadiene-1-ol, vinyl acetate,
acrylonitrile, styrene, p-aminostyrene, p-aminobenzylstyrene, sodium
styrenesulfonate, sodium-2-sulfoxyethylmethacrylate, vinyl pyridine,
aminoethylmethacrylate, 2-methacryloyloxytrimethylammonium chloride, and

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polyvinylidenes, such as polyfunctional cross-linkable monomers like for example N,N'-methylene-bis-acrylamide, ethylene glycol dimethacrylate, 2,2'-(p-phenylenedioxy)-diethyldimethacrylate, divinylbenzene, triallylamine and methylene-bis-(4-phenyl-isocyanate), including any desired combinations thereof.

5 Preferred polymers contain polyacrylic acid, polyethyleneimine, polymethacrylic acid, polymethylmethacrylate, polysiloxane, polydimethylsiloxane, polylactonic acid, poly([epsilon]-caprolactone), epoxy resins, poly(ethylene oxide), poly(ethylene glycol), and polyamides (e.g. Nylon) and the like or any arbitrary mixtures thereof. Preferred copolymers contain among others polyvinylidene-polyacrylonitrile,

10 polyvinylidene-polyacrylonitrile-polymethylmethacrylate, and polystyrene-polyacrylonitrile and the like or any desired mixtures thereof. Methods for manufacture of such micro spheres are published for example in Garner et al., U.S. Patent 4,179,546, Garner, U.S. Patent 3,945,956, Cohrs et al., U.S. Patent 4,108,806, Japan Kokai Tokkyo Koho 62 286534, British Patent 1,044,680, Kenaga et al., U.S.

15 Patent 3,293,114, Morehouse et al., U.S. Patent 3,401,475, Walters, U.S. Patent 3,479,811, Walters et al., U.S. Patent 3,488,714, Morehouse et al., U.S. Patent 3,615,972, Baker et al., U.S. Patent 4,549,892, Sands et al., U.S. Patent 4,540,629, Sands et al., U.S. Patent 4,421,562, Sands, U.S. Patent 4,420,442, Mathiowitz et al., U.S. Patent 4,898,734, Lencki et al., U.S. Patent 4,822,534, Herbig et al., U.S. Patent

20 3,732,172, Himmel et al., U.S. Patent 3,594,326, Sommerville et al., U.S. Patent 3,015,128, Deasy, Microencapsulation and Related Drug Processes, Vol. 20, Chapters. 9 and 10, pp. 195-240 (Marcel Dekker, Inc., N.Y., 1984), Chang et al., Canadian J of Physiology and Pharmacology, Vol 44, pp. 115-129 (1966), and Chang, Science, Vol. 146, pp. 524-525 (1964), and others and are incorporated

25 herein completely in accordance with the invention.

Other signal generating agents can in accordance with the invention be selected from the group of agents, which are transformed into signal generating agents in

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organisms by means of in-vitro or in-vivo cells, cells as a component of cell cultures, of in-vitro tissues, or cells as a component of multicellular organisms, such as for example fungi, plants or animals, in preferred embodiments from mammals like mice or humans. Such agents can be made available in the form of vectors for the

5 transfection of multicellular organisms, wherein the vectors contain recombinant nucleic acids for the coding of signal generating agents. In certain embodiments this is concerned with signal generating agents like metal binding proteins. It can be preferred to choose such vectors from the group of viruses for example from adeno

10 viruses, adeno virus associated viruses, herpes simplex viruses, retroviruses, alpha viruses, pox viruses, arena-viruses, vaccinia viruses, influenza viruses, polio viruses or hybrids of any of the above.

Further such signal generating agents are to be chosen in combination with delivery systems, in order to incorporate nucleic acids, which are suitable for coding for

15 signal generating agents, into the target structure. Especially preferred are virus particles for the transfection of mammalian cells, wherein the virus particle contains one or a plurality of coding sequence/s for one or a plurality of signal generating agents as described above. In these cases the particles are generated from one or a plurality of the following viruses: adeno viruses, adeno virus associated viruses,

20 herpes simplex viruses, retroviruses, alpha viruses, pox viruses, arena-viruses, vaccinia viruses, influenza viruses and polio viruses.

In further embodiments these signal generating agents are made available from colloidal suspensions or emulsions, which are suitable to transfect cells, preferably

25 mammalian cells, wherein these colloidal suspensions and emulsions contain those nucleic acids which possess one or a plurality of the coding sequence(s) for signal generating agents. Such colloidal suspensions or emulsions can contain macromolecular complexes, nano capsules, microspheres, beads, micelles, oil-in-

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water- or water-in-oil emulsions, mixed micelles and liposomes or any desired mixture of the above.

In further embodiments, cells, cell cultures, organized cell cultures, tissues, organs of
5 desired species and non-human organisms can be chosen which contain recombinant
nucleic acids having coding sequences for signal generating agents. In specific
embodiments organisms are selected from the groups: mouse, rat, dog, monkey, pig,
fruit fly, nematode worms, fish or plants or fungi. Further, cells, cell cultures,
organized cell cultures, tissues, organs of desired species and non-human organisms
10 can contain one or a plurality of vectors as described above.

Signal generating agents are preferably produced *in vivo* from the group of proteins
and made available as described above. Such agents are preferably directly or
indirectly signal producing, while the cells produce (direct) a signal producing
15 protein through transfection or produce a protein which induces (indirect) the
production of a signal producing protein. Preferably these signal generating agents
are detectable in methods such as MRI while the relaxation times T1, T2 or both are
altered and lead to signal producing effects which can be processed sufficiently for
imaging. Such proteins are preferably protein complexes, especially metalloprotein
20 complexes. Direct signal producing proteins are preferably such metalloprotein
complexes which are formed in the cells. Indirect signal producing agents are such
proteins or nucleic acids, for example, which regulate the homeostasis of iron
metabolism, the expression of endogenous genes for the production of signal
generating agents, and/or the activity of endogenous proteins with direct signal
25 generating properties, for example Iron Regulatory Protein (IRP), Transferrin
receptor (for the take-up of Fe), erythroid-5-aminobevulinate synthase (for the
utilization of Fe, H-Ferritin and L-Ferritin for the purpose of Fe storage). In specific
embodiments it can be preferred to combine both types of signal generating agents,

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that is direct and indirect, with each other, for example an indirect signal generating agent, which regulates the iron-homeostasis and a direct agent, which represents a metal binding protein.

- 5 In such embodiments, where preferably metal-binding polypeptides are selected as indirect agents, it is advantageous if the polypeptide binds to one or a plurality of metals which possess signal generating properties. Especially preferred are such metals with unpaired electrons in the *d* orbitals, such as for example Fe, Co, Mn, Ni, Gd etc., wherein especially Fe is available in high physiological concentrations in
- 10 organisms. It is moreover preferred, if such agents form metal-rich aggregates, for example crystalline aggregates, whose diameters are larger than 10 picometers, preferably larger than 100 picometers, 1 nm, 10 nm or specially preferred larger than 100 nm.
- 15 Preferred are such metal-binding compounds, which have sub-nanomolar affinities with dissociation constants of less than 10^{-15} M, 10^{-2} M or smaller. Typical polypeptides or metal-binding proteins are lactoferrin, ferritin, or other dimetallocooxymate proteins or the like, or so-called metal catcher with siderophoric groups, like for example haemoglobin. A possible method for
- 20 preparation of such signal generating agents, their selection and the possible direct or indirect agents which are producible *in vivo* and are suitable as signal generating agents was disclosed in WO 03/075747 and is incorporated herewith in accordance with the invention.
- 25 Another group of signal generating agents can be photophysically signal producing agents which consist of dyestuff-peptide-conjugates. Such dyestuff-peptide-conjugates are preferred which provide a wide spectrum of absorption maxima, for example polymethine dyestuffs, in particular cyanine-, merocyanine-, oxonol- and

squarilium dyestuffs. From the class of the polymethin dyestuffs the cyanine dyestuffs, e.g. the indole structure based indocarbo-, indodicarbo- and indotricarbocyanines, are especially preferred. Such dyestuffs can be preferred in specific embodiments, which are substituted with suitable linking agents and can be
5 functionalized with other groups as desired. In this connection see also DE 19917713, which is incorporated explicitly by reference.

In accordance with the invention, signal generating agents can be functionalized as desired. The functionalization by means of so-called "Targeting" groups is preferred
10 are to be understood, as functional chemical compounds which link the signal generating agent or its specifically available form (encapsulation, micelles, micro spheres, vectors etc.) to a specific functional location, or to a determined cell type, tissue type or other desired target structures. Preferably targeting groups permit the accumulation of signal-producing agents in or at specific target structures. Therefore
15 the targeting groups can be selected from such substances, which are principally suitable to provide a purposeful enrichment of the signal generating agents in their specifically available form by physical, chemical or biological routes or combinations thereof. Useful targeting groups to be selected can therefore be antibodies, cell receptor ligands, hormones, lipids, sugars, dextrane, alcohols, bile
20 acids, fatty acids, amino acids, peptides and nucleic acids, which can be chemically or physically attached to signal-generating agents, in order to link the signal-generating agents into/onto a specifically desired structure. In a first embodiment targeting groups are selected, which enrich signal-generating agents in/on a tissue type or on surfaces of cells. Here it is not necessary for the function, that the signal
25 generating agent be taken up into the cytoplasm of the cells. Peptides are preferred as targeting groups, for example chemotactic peptides are used to make inflammation reactions in tissues visible by means of signal generating agents; in this connection see also WO 97/14443, which is incorporated explicitly by reference.

Antibodies are also preferred, including antibody fragments, Fab, Fab2, Single Chain Antibodies (for example Fv), chimerical antibodies, and the like, as known from the prior art, moreover antibody-like substances, for example

5 so-called anticalines, wherein it is unimportant whether the antibodies are modified after preparation, recombinants are produced or whether they are human or non-human antibodies. It is preferred to choose from humanized or human antibodies, examples of humanized forms of non-human antibodies are chimerical immunoglobulines, immunoglobulin chains or fragments (like Fv, Fab, Fab',

10 F(ab'')₂ or other antigen-binding subsequences of antibodies, which partly contain sequences of non-human antibodies; humanized antibodies contain for example human immunoglobulines (receptor or recipient antibody), in which groups of a CDR (Complementary Determining Region) of the receptor are replaced through groups of a CDR of a non-human (spender or donor antibody), wherein the spender

15 species for example, mouse, rabbit or other has appropriate specificity, affinity, and capacity for the binding of target antigens. In a few forms the Fv framework groups of the human immunoglobulines are replaced by means of corresponding non-human groups. Humanized antibodies can moreover contain groups which either do not occur in either the CDR or Fv framework sequence of the spender or the recipient.

20 Humanized antibodies essentially comprise substantially at least one or preferably two variable domains, in which all or substantial components of the CDR components of the CDR regions or Fv framework sequences correspond with those of the non-human immunoglobulin, and all or substantial components of the FR regions correspond with a human consensus-sequence. In accordance with the

25 invention targeting groups of this embodiment can also be hetero-conjugated antibodies. Preferred function of the selected antibodies or peptides are cell surface markers or molecules, particularly of cancer cells, wherein here a large number of

known surface structures are known, such as HER2, VEGF, CA15-3, CA 549, CA 27.29, CA 19, CA 50, CA242, MCA, CA125, DE-PAN-2, etc., and the like.

Moreover, it is preferred to select targeting groups which contain the functional
 5 binding sites of ligands. Such can be chosen from all types, which are suitable for binding to any desired cell receptors. Examples of possible target receptors are, without limiting the choice, receptors of the group of insulin receptors, insulin-like growth factor receptor (e IGF-1 and IGF-2), growth hormone receptor, glucose transporters (particularly GLUT 4 receptor), transferrin receptor (transferrin),
 10 Epidermal Growth Factor receptor (EGF), low density lipoprotein receptor, high density lipoprotein receptor, leptin receptor, oestrogen receptor; interleukin receptors including IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-13, IL-15, and IL-17 receptor, VEGF receptor (VEGF), PDGF receptor (PDGF), Transforming Growth Factor receptor (including TGF-[alpha] and TGF-[beta]), EPO
 15 receptor (EPO), TPO receptor (TPO), ciliary neurotrophic factor receptor, prolactin receptor, and T-cell receptors.

It can be preferred to select hormone receptors, especially for hormones like steroidal hormones or protein- or peptide-based hormones, for example, however not limited
 20 thereto, epinephrines, thyroxines, oxytocine, insulin, thyroid-stimulating hormone, calcitonine, chorionic gonadotropine, corticotropine, follicle stimulating hormone, glucagons, leuteinizing hormone, lipotropine, melanocyte-stimulating hormone, norepinephrines, parathyroid hormone, Thyroid-Stimulating Hormone (TSH), vasopressin's, enkephalin, serotonin, estradiol, progesterone, testosterone, cortisone,
 25 and glucocorticoids. Receptor ligands include those which are on the cell surface receptors of hormones, lipids, proteins, glycol proteins, signal transducers, growth factors, cytokine, and other bio molecules. Moreover, targeting groups can be selected from carbohydrates with the general formula: $C_x(H_2O)_y$, wherein herewith

also monosaccharides, disaccharides and oligo- as well as polysaccharides are included, as well as other polymers which consist of sugar molecules which contain glycosidic bonds. Specially preferred carbohydrates are those in which all or parts of the carbohydrate components contain glycosylated proteins, including the monomers
5 and oligomers of galactose, mannose, fructose, galactosamine, glucosamine, glucose, sialic acid, and especially the glycosylated components, which make possible the binding to specific receptors, especially cell surface receptors. Other useful carbohydrates to be selected contain monomers and polymers of glucose, ribose, lactose, raffinose, fructose and other biologically occurring carbohydrates especially
10 polysaccharides, for example, however not exclusively, arabinogalactan, gum Arabica, mannan and the like, which are usable in order to introduce signal generating agents into cells. Reference is made in this connection to U.S. Patent 5,554,386 which is incorporated herewith in accordance with the invention.

15 Furthermore targeting groups can be selected from the lipid group, wherein also fats, fatty oils, waxes, phospholipids, glycolipids, terpenes, fatty acids and glycerides, especially triglycerides are included. Further included are eicosanoides, steroids, sterols, suitable compounds of which can also be hormones like prostaglandins, opiates and cholesterol and the like. In accordance with the invention all functional
20 groups can be selected as the targeting group, which possess inhibiting properties, such as for example enzyme inhibitors, preferably those which link signal generating agents into/onto enzymes.

In a second embodiment, targeting groups can be selected from a group of functional
25 compounds which make possible internalization or incorporation of signal generating agents in the cells, especially in the cytoplasm or in specific cell compartments or organelles, such as for example the cell nucleus. For example targeting group is preferred which contains all or parts of HIV-1 tat-proteins, their analogs and

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derivatized or functionally similar proteins, and in this way allows an especially rapid uptake of substances into the cells. As an example refer to Fawell et al., PNAS USA 91:664 (1994); Frankel et al., Cell 55:1189,(1988); Savion et al., J. Biol. Chem. 256:1149 (1981); Derossi et al., J. Biol. Chem. 269:10444 (1994); and Baldin et al.,
5 EMBO J. 9:1511 (1990), which are herewith explicitly incorporated in accordance with the invention.

Targeting groups can be further selected from the so-called Nuclear Localisation Signal (NLS), where under short positively charged (basic) domains are understood
10 which bind to specifically targeted structures of cell nuclei. Numerous NLS and their amino acid sequences are known including single basic NLS like that of the SV40 (monkey virus) large T Antigen (pro Lys Lys Lys Arg Lys Val), Kalderon (1984), et al., Cell, 39:499-509), the teinoic acid receptor-[beta] nuclear localization signal (ARRRRP); NFkB p50 (EEVQRKRQKL; Ghosh et al., Cell 62:1019 (1990); NFkB
15 p65 (EEKRKRTYE; Nolan et al., Cell 64:961 (1991), as well as others (see for example Boulikas, J. Cell. Biochem. 55(1):32-58 (1994), and double basic NLS's like for example xenopus (African clawed toad) proteins, nucleoplasmin (Ala Val Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys Leu Asp), Dingwall, et al., Cell, 30:449-458, 1982 and Dingwall, et al., J. Cell Biol., 107:641-849, 1988.
20 These are all incorporated herewith by reference in accordance with the invention. Numerous localization studies have shown that NLSs, which are built into synthetic peptides which normally do not address the cell nucleus or were coupled to reporter proteins, lead to an enrichment of such proteins and peptides in cell nuclei. In this connection exemplary references are made to Dingwall, and Laskey, Ann, Rev. Cell
25 Biol., 2:367-390, 1986; Bonnerot, et al., Proc. Natl. Acad. Sci. USA, 84:6795-6799, 1987; Galileo, et al., Proc. Natl. Acad. Sci. USA, 87:458-462, 1990. It can be especially preferred to select targeting groups for the hepatobiliary system, wherein

in U.S. Patents 5,573,752 and 5,582,814 corresponding groups are suggested. Both publications are included herein by reference.

THERAPEUTICALLY ACTIVE AGENTS

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In accordance with the invention at least one therapeutic agent is also chosen in addition to a signal generating agent. Therapeutic agents include all substances, which develop local and/or systemic physiological and/or pharmacological effects in animals, specially in mammals, for example, including in accordance with the invention all mammals like, however not exclusively, domestic animals like dogs and cats, agricultural beasts of burden like pigs, cattle, sheep, or goats, laboratory animals like mice, rats, primates like apes, chimpanzees etc., and humans. Therapeutic agents can be present in the composition or combination in accordance with the invention in crystalline, polymorphous or amorphous forms or any mixtures thereof. Useful therapeutically active ingredients can be chosen from a large number of therapeutically effective substances, for example, however not exclusively, from the group of enzyme inhibitors, hormones, cytokines, growth factors, receptor ligands, antibodies, antigens, ion-binding materials, among which are also to be numbered crown ethers and other chelating agents, substantially complementary nucleic acids, nucleic acid binding proteins including transcription factors, toxins, etc. Further useful materials include cytokines like erythropoietin (EPO), thrombopoietin (TPO), interleukin (including IL-1 through IL-17), insulin, insulin-like growth factors (including IGF-1 and IGF-2), epidermal growth factor (EGF), transforming growth factors (including TGF-[alpha] and TGF-[beta]), human growth hormone, transferrin, epidermal growth factor (EGF), Low density lipoprotein, high density lipoprotein, leptin, VEGF, PDGF, ciliary neurotrophic factor, prolactin, adrenocorticotrophic hormone (ACTH), calcitonin, human chorionic gonadotropin, cortisol, estradiol, follicle stimulating hormone (FSH), thyroid-stimulating hormone (TSH), leutinizing

hormone (LH), progesterone, testosterone, toxin including ricin, and all other materials which are listed in the publications Physician's Desk Reference, 58th Edition, Medical Economics Data Production Company, Montvale, N.J., 2004 and the Merck Index, 13th Edition (especially pages Ther-1 through Ther-29), wherein
5 both are explicitly incorporated herewith by reference.

In a preferred embodiment the therapeutically active substance is chosen from the group of active substances for the therapy of oncological diseases and cell or tissue changes. Useful therapeutic agents are for example however not exclusively anti-
10 neoplastically active substances, including alkylating agents like alkyl sulfonates (e.g. busulfane, improsulfane, piposulfane), aziridines (e.g. benzodepa, carboquone, meturedpa, uredepa); ethylene imines and methylmelamine (e.g. altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate, trimethylolmelamine); so-called nitrogen mustards (e.g. chlorambucil,
15 chlornaphazine, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethaminoxide hydrochloride, melphalan, novembichine, phenesterine, prednimustine, trofosfamide, uracil mustard); nitrosourea compounds (carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine); dacarbazine, mannomustine, mitobranitol, mitolactol; pipobromane; doxorubicine, and cis-platin
20 (including derivatives), or the like and their derivatives. In another preferred embodiment the therapeutically active substance is chosen from the group of antiviral and antibacterial active substances including aclacinomycine, actinomycine, anthramycine, azaserine, bleomycin, cuctinomycine, carubicine, carzinophiline, chromomycine, ductinomycine, daunorubicine, 6-diazo-5-oxn-1-norieucine,
25 duxorubicine, epirubicine, mitomycine, mycophenolic acid, nogalumycine, olivomycine, peplomycine, plicamycine, porfiromycine, puromycine, streptonigrine, streptozocine, tubercidine, ubenimex, zinostatine, zorubicine, the aminoglycosides or polyenes or macrolide antibiotics, and the like or their derivatives.

In a preferred embodiment the therapeutically active substance is selected from the group of radio-sensitizer drugs.

- 5 In a further preferred embodiment the therapeutically active substance is chosen from the group of steroidal active substances as well also as non-steroidal anti-inflammatory active substances.

- 10 In a further preferred embodiment the therapeutically active substance is chosen from active substances which relate to the angiogenesis, for example, however not exclusively, endostatin, angiostatin, interferones, platelet factor 4 (PF4), thrombospondine, transforming growth factor beta, the tissue inhibitors of metalloproteinase -1, -2 und -3 (TIMP-1, -2 and -3), TNP-470, marimastate, neovastate, BMS-275291, COL-3, AG3340, thalidomide, squalamine,
15 combrestastatin, SU5416, SU6668, IFN-[alpha], EMD121974, CAI, IL-12 and IM862, and the like or their derivatives.

- In a further preferred embodiment the therapeutically active substance is chosen from the group of the nucleic acids, also including oligonucleotides in addition to nucleic
20 acids and wherein at least two nucleotides are covalently linked with each other, for example, however not exclusively, in order to produce gene therapeutic or anti sense effects. Nucleic acids preferably contain phosphodiester linkages, wherein also those are included which are present as analogs with various backbones. Analogs can also contain as backbones for example, however not exclusively, phosphoramides
25 (Beaucage et al., Tetrahedron 49(10):1925 (1993) and the references given there; Letsinger, J. Org. Chem. 35:3800 (1970); Sprinzl et al., Eur. J. Biochem. 81:579 (1977); Letsinger et al., Nucl. Acids Res. 14:3487 (1986); Sawai et al, Chem. Lett. 805 (1984), Letsinger et al., J. Am. Chem. Soc. 110:4470 (1988); and Pauwels et al.,

Chemica Scripta 26:141 (1986)), phosphorothioates (Mag et al., Nucleic Acids Res. 19:1437 (1991); and U.S. Patent 5,644,048), phosphorodithioates (Briu et al., J. Am. Chem. Soc. 111:2321 (1989), O-methylphosphoroamidite compounds (see Eckstein, Oligonucleotides and Analogues: A Practical Approach, Oxford University Press),
5 and Peptide-Nucleic Acid Backbones and their Compounds (see Egholm, J. Am. Chem. Soc. 114:1895 (1992); Meier et al., Chem. Int. Ed. Engl. 31:1008 (1992); Nielsen, Nature, 365:566 (1993); Carlsson et al., Nature 380:207 (1996), wherein the references given herewith are incorporated explicitly in accordance with the invention. Other analogs contain those with ionic backbones, see (Denpcy et al.,
10 Proc. Natl. Acad. Sci. USA 92:6097 (1995), or non-ionic backbones, see U.S. Patents 5,386,023, 5,637,684, 5,602,240, 5,216,141 and 4,469,863; Kiedrowshi et al., Angew. Chem. Intl. Ed. English 30:423 (1991); Letsinger et al., J. Am. Chem. Soc. 110:4470 (1988); Letsinger et al., Nucleosides & Nucleotides 13:1597 (1994); Chapter 2 and 3, ASC Symposium Series 580, "Carbohydrate Modifications in
15 Antisense Research", Ed. Y. S. Sanghui and P. Dan Cook; Mesmaeker et al., Bioorganic & Medicinal Chem. Lett. 4:395 (1994); Jeffs et al., J. Biomolecular NMR 34:17 (1994); Tetrahedron Lett. 37:743 (1996), and Non-Ribose Backbones, incorporated the ones which are described in U.S. Patents 5,235,033 and 5,034,506, and Chapter 6 and 7, ASC Symposium Series 580, "Carbohydrate Modifications in
20 Antisense Research", Ed. Y. S. Sanghui and P. Dan Cook. The references cited are incorporated explicitly in accordance with the invention. Nucleic acids having one or a plurality of carbocyclic sugars are likewise useable as nucleic acids in accordance with the invention, see Jenkins et al., Chem. Soc. Rev. (1995) pp 169-176, as well as others which are described in Rawls, C & E News June 2, 1997, page 35, and are
25 explicitly incorporated herewith. In addition to the selection of useable nucleic acids and nucleic acid analogs known from the prior art, any desired mixtures of naturally occurring nucleic acids and nucleic acid analogs or mixtures of nucleic acid analogs can also be used.

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In one embodiment the therapeutically active substance is chosen from the group of metal ion complexes, as generally described in PCT US95/16377, PCT US95/16377, PCT US96/19900, PCT US96/15527 and incorporated completely herewith by
5 reference, wherein such agents reduce or inactivate the bioactivity of their target molecules, preferably proteins, for example, however not exclusively, enzymes.

Preferred therapeutically active substances are further antimigratory, antiproliferative or immuno-suppressive, antiinflammatory and re-endothelialising active substances
10 such as for example, however not exclusively everolimus, tacrolimus, sirolimus, mycophenolate mofetil, rapamycin, paclitaxel, actinomycin D, angiopeptin, batimastat, oestradiol, VEGF, statins and their derivatives and analogs.

Especially preferred are active substances or active substance combinations, which
15 are selected from heparin, synthetic heparin- analogs (e.g. fondaparinux), hirudin, antithrombin III, drotrecogin alpha; fibrinolytics like alteplase, plasmin, lysokinas, factor XIIa, prourokinase, urokinase, anistreplase, streptokinase; thrombozyte aggregations inhibitors like acetylsalicylic acid, ticlopidine, clopidogrel, abciximab, dextran; cortico-steroids like alclometasone, amcinonide,
20 augmented betamethasone, beclomethasone, betamethasone, budesonide, cortisone, clobetasol, clocortolone, desonide, desoximetasone, dexamethasone, flucinolone, fluocinonide, flurandrenolide, flunisolide, fluticasone, halcinonide, halobetasol, hydrocortisone, methylprednisolone, mometasone, prednicarbate, prednisone, prednisolone, triamcinolone; so-called non-steroidal anti-inflammatory drugs like
25 diclofenac, diflunisal, etodolac, fenoprofen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, meclofenamate, mefenamic acid, meloxicam, nabumetone, naproxen, oxaprozin, piroxicam, salsalate, sulindac, tolmetin, celecoxib, rofecoxib; zyto-statiks like alkaloids and podophyllum toxins like vinblastine, vincristine;

alkylating agents like nitroso urea, nitrogen lacking analogs; cytotoxic antibiotics like daunorubicin, doxorubicin and other anthracyclines and related substances, bleomycin, mitomycin, anti-metabolite like folic acid, purine or pyrimidine analogs; paclitaxel, docetaxel, sirolimus; platinum compounds like carboplatinum, cisplatinum or oxali-platinum; amsacrine, irinotecan, imatinib, topotecan, interferon alpha 2a, interferon alpha 2b, hydroxycarbamide, miltefosine, pentostatin, porfimer, aldesleucine, bexarotene, tretinoin; antiandrogens, and antioestrogens; antiarrhythmics, especially antiarrhythmics of Class I like antiarrhythmics of the chinidine type, e.g., chinidine, dysopyramide, ajmaline, prajmaliumbitartrate, detajmumbitartrate; antiarrhythmics of the lidocaine type, e.g., lidocaine, mexiletine, phenytoine, tocainide; antiarrhythmics of Class I C, e.g., propafenone, flecainide (acetate); antiarrhythmics of Class II, beta-receptors blockers like metoprolol, esmolol, propranolol, metoprolol, atenolol, oxprenolol; antiarrhythmics of Class III like amiodarone, sotalol; antiarrhythmics of Class IV like diltiazem, verapamil, gallopamil; other antiarrhythmics like adenosine, orciprenaline, ipratropium bromide; agents for stimulation of angiogenesis in the myocardia like Vascular Endothelial Growth Factor (VEGF), Basic Fibroblast Growth Factor (bFGF), non-viral DNA, viral DNA, endothelial growth factors: FGF-1, FGF-2, VEGF, TGF; antibodies, monoclonal antibodies, anticaline; stem cells, Endothelial Progenitor Cells (EPC); digitalisglycosides like acetyldigoxine/metildigoxine, digitoxin, digoxin; heart glycosides like quabaine, proscillaridine; antihypertension drugs like central-functioning antiadrenal energy substances, e.g., centrally active antiadrenergic substances, e.g., methyldopa, imidazoline receptor-agonists; calcium channel blockers of the dihydropyridine type like nifedipine, nitrendipine; ACE-inhibitors: quinaprilate, cilazapril, moexipril, trandolapril, spirapril, imidapril, trandolapril; angiotensin-II-antagonists: candesartane cilexetil, valsartane, telmisartane, olmesartane medoxomil, eprosartane; peripherally operating alpha-Receptor blockers like prazosin, urapidil, doxazosin, bunazosin, terazosin, indoramine; vaso-dilatators

- like dihydralazine, diisopropylaminedichloracetate, minoxidil, nitroprussid sodium; other anti-hypertension drugs like indapamide, co-dergocrine mesilate, dihydroergotoxinmethanesulfonate, cicletanin, bosentane, fludrocortisone; phosphodiesterase inhibitors like milrinone, enoximone and antihypotonics,
- 5 especially like adrenal and dopaminergic especially adrenergic and dopaminergic substances like dobutamine, epinephrine, etilefrin, norfenefrin, nor epinephrine, oxilofrin, dopamine, midodrin, pholedrin, ameziniummetil; and partial adrenoceptor agonists like dihydroergotamine; fibronectine, polylysine, ethylene vinyl acetate, inflammatory cytokines like: TGF β , PDGF, VEGF, bFGF, TNF α , NGF, GM-CSF,
- 10 IGF-a, IL-1, IL-8, IL-6, Growth Hormone; as well as adhesive substances like cyanoacrylates, beryllium, silica; and growth factors like erythropoietin, hormones like corticotropine, gonadotropine, somatropin, thyrotrophin, desmopressin, terlipressin, oxytocin, cetrorelix, corticorelin, leuprorelin, triptorelin, gonadorelin, ganirelix, buserelin, nafarelin, goserelin, as well as regulating peptides like
- 15 somatostatin, octreotide; bone and cartilage stimulating peptides, Bone Morphogenetic Proteins (BMPs), especially recombinant BMP's like e.g., recombinant human BMP-2 (rhBMP-2)), bisphosphonates (e.g., risedronate, pamidronate, ibandronate, zoledronic acid, clodronin acid, etidronic acid, alendronic acid, tiludronic acid), fluorides like disodiumfluorophosphate, sodium fluoride;
- 20 calcitonin, dihydrotachystyrene; growth factors and cytokines like Epidermal Growth Factor (EGF), Platelet-Derived Growth Factor (PDGF), Fibroblast Growth Factors (FGFs), Transforming Growth Factors-b TGFs-b), Transforming Growth Factor-a (TGF-a), Erythropoietin (Epo), Insulin-Like Growth Factor-I (IGF-I), Insulin-Like Growth Factor-II (IGF-II), Interleukin-1 (IL-1), Interleukin-2 (IL-2), Interleukin-6
- 25 (IL-6), Interleukin-8 (IL-8), Tumor Necrosis Factor-a (TNF-a), Tumor Necrosis Factor-b (TNF-b), Interferon-g (INF-g), Colony Stimulating Factors (CSFs); monocyte chemotactic protein, fibroblast stimulating factor 1, histamine, fibrin or fibrinogen, endothelin-1, angiotensin II, collagens, bromocriptin, methylsergide,

- methotrexate, carbon tetrachloride, thioacetamide, and ethanol; further silver(ions), titanium dioxide, antibiotics and anti-infectives like especially β -lactam-antibiotics, e.g. β -lactamase-sensitive penicillins like benzyl penicillins (penicillin G), phenoxymethyl penicillin (penicillin V); β -lactamase-resistant penicillins like
- 5 aminopenicillins like amoxicillin, ampicillin, bacampicillin; acylamino penicillins like mezlocillin, piperacillin; carboxypenicillins, cephalosporines like cefazolin, cefuroxim, cefoxitin, cefotiam, cefaclor, cefadroxil, cefalexin, loracarbef, cefixim, cefuroximaxetil, ceftibuten, cefpodoximproxetil; aztreonam, ertapenem, meropenem; β -lactamase inhibitors like sulbactam, sultamicillintosilate; tetracyclines like
- 10 doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline; aminoglycosides like gentamicin, neomycin, streptomycin, tobramycin, amikacin, netilmicin, paromomycin, framycetin, spectinomycin; macrolide antibiotics like azithromycin, clarithromycin, erythromycin, roxithromycin, spiramycin, josamycin; lincosamides like clindamycin, lincomycin, gyrase inhibitors like fluoroquinolone
- 15 like ciprofloxacin, ofloxacin, moxifloxacin, norfloxacin, gatifloxacin, enoxacin, fleroxacin, levofloxacin; chinolones like pipemide acid; sulfonamides, trimethoprim, sulfadiazine, sulfalene; glycopeptide antibiotics like vancomycin, teicoplanin; polypeptide antibiotics like polymyxine like colistin, polymyxin-B, nitroimidazole derivatives like metronidazol, tinidazol; aminochinolones like chloroquin, mefloquin,
- 20 hydroxychloroquin; biguanides like proguanil; chinin alkaloids and diaminopyrimidine like pyrimethamine; amphenicoles like chloramphenicol; rifabutin, dapson, fusidinic acid, fosfomycin, nifuratel, telithromycin, fusafungine, fosfomycine, pentamidindiisethionate, rifampicin, taurolidine, atovaquon, linezolid; virustatics like acyclovir, gancyclovir, famcyclovir, foscarnet, inosine-(dimepranol-
- 25 4-acetamidobenzoate), valgancyclovir, valacyclovir, cidofovir, brivudine; antiretroviral active substances (nucleoside analogous reverse transcriptase inhibitors und derivatives) like lamivudine, zalcitabine, didanosine, zidovudine, tenofovir, stavudine, abacavir; non-nucleoside analogs reverse-transcriptase inhibitors:

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amprenavir, indinavir, saquinavir, lopinavir, ritonavir, nelfinavir; amantadine, ribavirine, zanamivir, oseltamivir and lamivudine, and the like as well as arbitrary combinations and mixtures thereof.

- 5 Moreover therapeutically active substances can be selected from microorganisms, plant or animal cells including human cells or cell cultures and tissues, especially recombinant cells or organized cells or tissues, preferably from mammals, especially preferred heterologic or autologic cells or tissues, or transfected cells, which express and release physiological or pharmacologically active substances. Stem cells,
10 primary cells as well as progenitor cells of differentiated primary cells or arbitrary mixtures thereof are to be preferred. It can moreover be preferred to use cells or organized cells or tissues as therapeutic agents, which are not transfixed and/or altered by means of gene technology.

15 MULTIFUNCTIONAL AGENTS

- In accordance with the invention various signal generating agents can be coupled with each other to bifunctional, trifunctional or multifunctional signal generating agents, while they are built from several functional units which are linked with each
20 other. Thereby it is possible to link any desired different signal generating agents with each other, so that complex signal generating agents combine different signal generating properties in a conjugate. Also such conjugated signal generating agents can additionally contain targeting groups or therapeutic active substances, which are joined as therapeutic groups to the conjugated complex. Therefore, in accordance
25 with the invention, bifunctional signal generating agents consist of a signal generating agent and a further agent having different signal generating properties, for example, however not exclusively, of a paramagnetic agent for the signal generation by means of MRI and a coupled fluorescence marker as disclosed for example in

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WO 04/026344, or of a paramagnetic and a diamagnetic group coupled in the MRI signal generating agent as disclosed in EP 1105162 or WO 00/09170; further dimeric signal generating agents of a super paramagnetic or ferromagnetic and X-ray contrast components as disclosed in U.S. Patent 5,346,690, or a paramagnetic and iodated
5 component composite agent for MRI and X-rays, as disclosed in U.S. Patent 5,242,683; the references are herewith incorporated in accordance with the invention.

In accordance with the invention bifunctional signal generating agents also consist of a signal generating agent and a therapeutically active substance or of a signal
10 generating agent and a targeting group. Examples for combined signal-producing agents with therapeutically active substances are disclosed in U.S. Patent 6,207,133, U.S. Patent 6,479,033, German Patent 10151791, Canadian Patent 1336164, WO 02/051301, WO 97/05904, European Patent 0458079, German Patent 4035187, WO 04/071536, U.S. Patent 6,811,766, WO 04/080483 and others and are incorporated
15 herewith by reference explicitly. Examples for combined signal-generating agents with targeting groups are disclosed in U.S. Patent 6,232,295, CN 1224622, WO 99/20312, WO 04/071536, U.S. Patent 6,207,133, WO 97/36619, U.S. Patent 6,652,835, WO 03/011115, WO 04/080483 and others and incorporated herewith by reference explicitly

20 Trifunctional signal generating agents comprise in accordance with the invention at least one signal generating component and a further signal generating component or a therapeutically active component or a targeting group and a further signal generating component or a therapeutically active agent or a targeting group.

25 Multifunctional signal generating agents can be correspondingly selected from a trifunctional signal generating agent having at least one other component which can be chosen arbitrarily. Especially, it is disclosed in U.S. Ser. No. 08/690,612, how

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multifunctional or multimeric signal-generating agents are manufactured in principle, wherein this is explicitly incorporated.

- The bi, tri and multifunctional signal generating agents can be present corresponding to the prior art entirely or partially as covalently or not covalently bonded macromolecules, as micelles or micro spheres, encapsulated in liposomes or encapsulated in polymers or bound covalently in polymers. For covalent bonds substituents in the form of functional groups in accordance with the prior art are coupled to the individual components, to be chosen for example are amino, carboxyl, oxo or thiol groups. These groups can be linked with each other directly or by means of a linker. Prior art linkers have been described many times, for example homo or heterofunctional linkers as described in Pierce Chemical Company catalogue, technical section on cross-linkers, pages 155-200, (1994), and incorporated herewith by reference. Preferred linkers include, however not exclusively, alkyl groups including substituted alkyl groups and alkyl groups with heteroatom groups, short chain alkyl groups esters, amides, amines, epoxy groups, nucleic acid, peptide, ethylene glycol, hydroxyl, succinimidyl, maleicidyl, biotin, aldehyde or nitrilotriacetate groups and with their derivatives.
- In accordance with the invention, mono, bi, tri or multifunctional signal generating agents can be linked non-covalently or partially or completely covalently, be encapsulated in micelles wherein the micelles can have a diameter of 2 nm to 800 nm, preferably from 5 to 200 nm, especially preferred from 10 to 25 nm. The size of the micelles is, without being tied to an established theory, dependent on the number of hydrophobic and hydrophilic groups, on the molecular weight of the signal generating agents used and the aggregation number. In aqueous solutions the use of branched or unbranched amphiphilic substances present as monomer or oligomer or polymer is especially preferred, in order to achieve encapsulation of the signal

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generating agents. The hydrophobic nucleus of the micelles hereby contains a multiplicity of hydrophobic groups, preferably between 1 and 200 according to the desired setting of the micelle size. Signal generating agents, targeting groups of the therapeutic agents can in accordance with the invention also be present in the
5 micelles to be provided partially linked covalently with each other.

Hydrophobic groups preferably comprise hydrocarbon groups or residues or silicone, for example polysiloxane chains. Moreover they can preferably be chosen from hydrocarbon-based monomers, oligomers and polymers, or from lipids or
10 phospholipids or any desired combinations, specially Glyceryl esters such as phosphatidyl ethanolamine, phosphatidyl cholines, or polyglycolides, polylactides, polymethacrylate, polyvinylbutylether, polystyrene, polycyclopentadienylmethylnorbornene, polyethylenepropylene, polyethylene, polyisobutylene, polysiloxane. Further for the encapsulation in micelles hydrophilic
15 polymers can also be selected, especially preferred polystyrene sulfonic acid, poly-N-alkylvinylpyridinium halides, poly(meth)acrylic acid, polyamino acids, poly-N-vinylpyrrolidone, polyhydroxyethylmethacrylate, polyvinyl ether, polyethylene glycol, polypropylene oxide, polysaccharides like agarose, dextran, starch, cellulose, amylose, amylopectin, or polyethylene glycol or polyethylene imines of arbitrary
20 molecular weight, according to the desired micelle property. Further, mixtures of hydrophobic or hydrophilic polymers can also be used or such lipid-polymer compounds employed. In a further special embodiment the polymer is used conjugated as a block copolymer, wherein hydrophilic as well as hydrophobic polymers or any desired mixtures thereof as 2-, 3- or multi-block copolymers can be
25 selected.

Such signal generating agents encapsulated in micelles and other functional components can be functionalized further, while linkers are attached at any desired

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positions of the micelle, preferably amino, thiol, carboxyl, hydroxyl, succinimidyl, maleimidyl, biotin, aldehyde or nitrilotriacetate groups, to which in accordance with the prior art further molecules or compounds can be bonded chemically covalent or non-covalent. Here especially biological molecules such as proteins, peptides, amino
5 acids, polypeptides, lipoproteins, glycosaminoglycane, DNA, RNA or similar bio molecules are preferred, in particular.

In accordance with the invention, mono-, bi-, tri-, or multi functional signal-generating agents, non-covalently or partially or completely covalently linked, also
10 in micro spheres and liposomes, are provided. Preferred micro spheres in a size of < 1000 μm can preferably be chosen from biocompatible synthetic polymers or copolymers, which consist of monomers, dimers or oligomers or other preferred pre-polymeric precursors of the following polymerizable substances: acrylic acid, methacrylic acid, ethyleneimine, crotonic acid, acryl amide, ethylacrylate,
15 methylmethacrylate, 2-hydroxyethylmethacrylate (HEMA), lactonic acid, glycolic acid, [epsilon]-caprolactone, acrolein, cyanoacrylate, bisphenol-A, epichlorhydrin, hydroxyalkylacrylate, siloxane, dimethylsiloxane, ethylene oxide, ethylene glycol, hydroxyalkylmethacrylate, N-substituted acryl amide, N-substituted methacrylamide, N-vinyl-2-pyrrolidone, 2,4-pentadiene-1-ol, vinyl acetate, acrylonitrile, styrene, p-aminostyrene, p-aminobenzylstyrene, sodium styrenesulfonate, sodium 2-sulfoxyethylmethacrylate, vinyl pyridine, aminoethylmethacrylate, 2-methacryloyloxytrimethylammonium chloride, also polyvinylidene, or polyfunctional cross-linked monomers such as for example N,N'-methylene-bis-acrylamide, ethylene glycol dimethacrylate, 2,2'-(p-phenylenedioxy)-diethyl-
20 dimethacrylate, divinylbenzene, triallylamine or methylene-bis-(4-phenylisocyanate), and the like or their derivatives or copolymers including any combinations hereof. Preferred polymers include polyacrylic acid, polyethyleneimine, polymethacrylic acid, polymethylmethacrylate, polysiloxane, polydimethylsiloxane, polylactonic

acid, poly([epsilon]-caprolactone), epoxy resins, poly(ethylene oxide), poly(ethylene glycol), and polyamide (nylon) and the like or their derivatives or copolymers or any desired mixtures thereof. Preferred copolymers include among others polyvinylidene polyacrylonitrile, polyvinylidene polyacrylonitrile polymethylmethacrylate, or
5 polystyrene polyacrylonitrile and the like or their derivatives or any mixtures thereof. Methods for the manufacture of such micro spheres are for example disclosed in Garner et al., U.S. Patent 4,179,546, Garner, U.S. Patent 3,945,956, Cohrs et al., U.S. Patent 4,108,806, Japan Kokai Tokkyo Koho 62 286534, British Patent 1,044,680, Kenaga et al., U.S. Patent 3,293,114, Morehouse et al., U.S. Patent 3,401,475,
10 Walters, U.S. Patent 3,479,811, Walters et al., U.S. Patent 3,488,714, Morehouse et al., U.S. Patent 3,615,972, Baker et al., U.S. Patent 4,549,892, Sands et al., U.S. Patent 4,540,629, Sands et al., U.S. Patent 4,421,562, Sands, U.S. Patent 4,420,442, Mathiowitz et al., U.S. Pat. No. 4,898,734, Lencki et al., U.S. Patent 4,822,534, Herbig et al., U.S. Patent 3,732,172, Himmel et al., U.S. Patent 3,594,326,
15 Sommerville et al., U.S. Patent 3,015,128, Deasy, Microencapsulation and Related Drug Processes, Vol. 20, Chapters. 9 and 10, pp. 195-240 (Marcel Dekker, Inc., N.Y., 1984), Chang et al., Canadian J of Physiology and Pharmacology, Vol 44, pp. 115-129 (1966), and Chang, Science, Vol. 146, pp. 524-525 (1964), and others and are completely incorporated by reference herewith in accordance with the invention.

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In accordance with the invention, mono, bi, tri or multifunctional signal generating agents, non-covalent or completely covalent linked are made available in liposomes partially or. It is preferred to choose from the group of anionic or cationic lipids as already explained in the appropriate section.

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Signal-generating agents, present as mono, bi, tri or multifunctional agents can also be linked with polymers. A general overview of methods in this connection is to be found in PCT US95/14621 and U.S. Ser. No. 08/690,612, both are incorporated

herewith by reference explicitly. In general signal-generating agents can, for example, be linked with polymers, while chemical groups are available, which allow a bond to be made from the signal-generating agents to the polymer or polymer mixture selected. Polymers are to be understood, as compounds which contain at least two or three sub-units which are covalently linked to each other. At least one part of a monomer sub-unit contains one or a plurality of functional groups which allow covalent bonding to the signal-generating agent. In a few embodiments coupling groups are used in order to link the monomeric sub-groups with the signal-generating agents. A multiplicity of polymers are suitable for this according to the prior art. Preferred polymers include, however not exclusively, functionalized styrene, like amino styrene, functionalized dextrane and polyamino acids. Preferred polymers are polyamino acids, (poly-D-amino acids as well as poly-L-amino acids), for example polylysine, and polymers which contain lysine or other suitable amino acids. Other useful polyamino acids are polyglutamic acids, polyaspartic acid, copolymers of lysine and glutamine or aspartic acid, copolymers of lysine with alanine, tyrosine, phenylalanine, serine, tryptophan and/or proline.

The polymers used can principally be selected from functionalized or non-functionalized polymers like for example, however not exclusively, thermosets, thermoplastics, synthetic rubbers, extrudable polymers, injection molding polymers, moldable polymers, and the like or mixtures, additionally as components of any composites. Further, additives can be chosen which improve the compatibility of the components used for producing the materials, for example coupling agents like silanes, surfactants or fillers, like organic or inorganic fillers.

In one embodiment the polymer is selected from polyacrylates like polymethacrylate, or from unsaturated polyesters, from saturated polyesters, a polyolefin (for example polyethylene, polypropylene, polybutylene, and similar), an alkyd resin, an epoxy-

polymer, a polyamide, a polyimide, polyetherimide, a polyamideimide, a polyesterimide, a polyesteramideimide, polyurethanes, polycarbonates, polystyrenes, polyphenol, polyvinylester, polysilicone, polyacetal, cellulose acetates, polyvinyl chlorides, polyvinyl acetates, polyvinyl alcohols, polysulfones, polyphenylsulfones, polyethersulfones, polyketones, polyetherketones, polyetheretherketones, polyetherketoneketones, polybenzimidazoles, polybenzoxazoles, polybenzthiazoles, polyfluorocarbons, polyphenylenether, polyarylates, cyanatoester-polymers, copolymers from two or more of those named, and the like.

- 10 Usable polymers are in particular acrylics, so preferred are monoacrylates, diacrylates, triacrylates, tetraacrylates, pentacrylates, and the like. Examples of polyacrylates are polyisobornylacrylate, polyisobornylmethacrylate, polyethoxyethoxyethylacrylate, poly-2-carboxyethylacrylate, polyethylhexylacrylate, poly-2-hydroxyethylacrylate, poly-2-phenoxyethylacrylate, poly-2-phenoxyethylmethacrylate, poly-2-ethylbutylmethacrylate, poly-9-anthracenylmethylmethacrylate, poly-4-chlorophenylacrylate, polycyclohexylacrylate, polydicyclopentenylloxyethylacrylate, poly-2-(N,N-diethylamino)ethylmethacrylate, poly-dimethylaminoeopentylacrylate, poly-caprolactone 2-(methacryloxy)ethylester, or polyfurfurylmethacrylate, poly(ethylene glycol)methacrylate, polyacrylic acid and poly(propylene glycol)methacrylate.

Examples of preferred usable diacrylates, from which polyacrylates can be produced, are 2,2-bis(4-methacryloxyphenyl)propane, 1,2-butanedioldiacrylate, 1,4-butanedioldiacrylate, 1,4-butanedioldimethacrylate, 1,4-cyclohexanedioldimethacrylate, 1,10-decanedioldimethacrylate, diethyleneglycoldiacrylate, dipropyleneglycoldiacrylate, dimethylpropanedioldimethacrylate, triethyleneglycoldimethacrylate, tetraethyleneglycoldimethacrylate, 1,6-hexanedioldiacrylate, neopentylglycoldiacrylate, polyethyleneglycoldimethacrylate,

tripropylene glycoldiacrylate, 2,2-bis[4-(2-acryloxyethoxy)phenyl]propane, 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane, bis(2-methacryloxyethyl)N,N-1,9-nonylenebiscarbamate, 1,4-cyclohexanedimethanoldimethacrylate, and diacrylic urethane oligomers.

5

Examples of triacrylates, which can be used for the manufacture of polyacrylates are preferably tris(2-hydroxyethyl)isocyanuratetrimethacrylate, tris(2-hydroxyethyl)isocyanuratetriacrylate, trimethylolpropanetrimethacrylate, trimethylolpropanetriacrylate or pentaerythritoltriacrylate. Examples of preferred
 10 tetraacrylates are pentaerythritoltetraacrylate, ditrimethylopropane tetraacrylate, or ethoxylated pentaerythritoltetraacrylate. Examples for pentaacrylates are dipentaerythritolpentaacrylate and pentaacrylate-ester.

Polyacrylates also comprise other aliphatic unsaturated organic compounds such as
 15 for example polyacrylamides and unsaturated polyesters from condensation reactions of unsaturated dicarboxylic acids and diols, and vinyl compounds, but also compounds with terminal double bonds. Examples for vinyl compounds are N-vinylpyrrolidone, styrene, vinyl-naphthalene or vinylphthalimide. To the particularly preferred methacrylamide derivatives belong N-alkyl or N-alkylene-substituted or
 20 unsubstituted (meth)acryl amide, like for example acryl amide, methacrylamide, N-methacrylamide, N-methylmethacrylamide, N-ethylacrylamide, N,N-dimethylacrylamide, N,N-dimethylmethacrylamide, N,N-diethylacrylamide, N-ethylmethacrylamide, N-methyl-N-ethylacrylamide, N-isopropylacrylamide, N-n-propylacrylamide, N-isopropylmethacrylamide, N-n-propylmethacrylamide, N-
 25 acryloyloylpyrrolidine, N-methacryloylpyrrolidine, N-acryloylpiperidine, N-methacryloylpiperidine, N-acryloylhexahydroazepine, N-acryloylmorpholine or N-methacryloylmorpholine.

Other useful polymers in accordance with the invention are unsaturated and saturated polyesters, particularly also including alkyd resins. The polyesters can contain polymeric chains, a various number of saturated or aromatic dibasic acids and anhydrides. Further epoxy resins, which can be used as monomers, oligomers or
5 polymers, especially those which contain one or a plurality of oxiran rings, have an aliphatic, aromatic or mixed aliphatic-aromatic molecular structures, or exclusively non benzoides, thus aliphatic or cycloaliphatic structures with or without substituents like halogens, ester groups, ether groups, sulfonate groups, siloxane groups, nitro groups or phosphate groups or any combinations thereof. Especially preferred are
10 epoxy resins of the glycidyl-epoxy type, for example with diglycidylether groups of bisphenol-A. Especially preferred moreover are amino-derivatized epoxy resins, tetraglycidyl-diaminodiphenylmethane, triglycidyl-p-aminophenol, triglycidyl-m-aminophenol or triglycidylaminocresol and their isomers, phenol-derivatized epoxy resins like for example bisphenol-A-epoxy resins, bisphenol-F-epoxy resins,
15 bisphenol-S-epoxy resins, phenol-novolak epoxy resins, cresol-novolak epoxy resins or resorcinol epoxy resins, or alicyclic epoxy resins. Moreover halogenated epoxy resins, glycidylethers of polyhydric phenols, diglycidylethers of bisphenol A, glycidylethers of phenol-formaldehyde Novolak resins and resorcinol-diglycidylethers, as well as other epoxy resins, as described in U.S. Patent 3,018,262
20 and incorporated herewith by reference explicitly. In accordance with the invention the choice is not restricted to the examples mentioned alone; in particular mixtures of two or a plurality of epoxy resins can also be chosen as well as mono-epoxy components. The chosen epoxy resins also include UV-cross-linkable and cycloaliphatic resins.

25

Preferred polymers are also polyamides (nylons) like, for example, aliphatic or aromatic polyamides among others also in specific embodiments nylon-6- (polycaprolactam), nylon 6/6 (polyhexamethylenedipamide), nylon 6/10, nylon

6/12, nylon 6/T (polyhexamethylene terephthalamide), nylon 7 (polyenanthamide), nylon 8 (polycapryllactam), nylon 9 (polypelargonamide), nylon 10, nylon 11, nylon 12, nylon 55, nylon XD6 (poly meta-xylylene adipamide), nylon 6/I, polyalanine.

- 5 Further polymers, however not restricted thereto which are preferably employed are polyimides, polyetherimides, polyamideimides, polyesterimides, polyesteramideimides.

- In a specific embodiment conductive polymers are selected, preferably from
- 10 saturated or unsaturated polyparaphenylenevinylene, polyparaphenylene, polyaniline, polythiophene, polyazines, polyfuranes, polypyrroles, polyselenophene, poly-p-phenylenesulfide, polyacetylene either as monomers, oligomers or polymers, in any combination and mixtures with other monomers, oligomers or polymers or copolymers of the monomers named above. Especially preferred contain one or a
- 15 plurality of organic, for example alkyl or aryl radicals or similar, or inorganic radicals, such as for example silicon or germanium or the like, or any mixtures therefrom. Preferred are conducting or semi conducting polymers with resistivities between 10^{12} and 10^5 Ohm · cm. It can be especially preferred to choose such polymers in which complexed metal salts are contained which is why polymers are to
- 20 be preferred which contain nitrogen, oxygen, sulfur or halides or unsaturated double bonds or triple bonds, and others which are suitable for complex formation. For example, without restricting selection the of suitable polymers thereto, elastomers like polyurethanes and rubbers, adhesive polymers and plastics. Preferred metal salts include transition metal halides such as CuCl_2 , CuBr_2 , CoCl_2 , ZnCl_2 , NiCl_2 , FeCl_2 ,
- 25 FeBr_2 , FeBr_3 , CuI_2 , FeCl_3 , FeI_3 , or FeI_2 , furthermore salts like $\text{Cu}(\text{NO}_3)_2$, metal lactates, metal glutamates, metal succinates, metal tartrates, metal phosphates, metal oxalates, LiBF_4 , and $\text{H}_4\text{Fe}(\text{CN})_6$ and the like.

Furthermore biocompatible, here biodegradable, polymers are especially preferred, for example, however not exclusively, collagens, albumin, gelatin, hyaluronic acid, starch, cellulose (methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulose phthalate; further casein, dextran, polysaccharides, fibrinogen, poly(D,L-lactide), poly(D,L-lactide-coglycolide), poly(glycolide), poly(hydroxybutylate), poly(alkyl carbonate), poly(orthoesters), polyesters, poly(hydroxyvaleric acid), polydioxanone, poly(ethyleneterephthalate), poly(malic acid), poly(tartronic acid), polyanhydride, polyphosphazene, poly(amino acids), and all their copolymers or any mixtures.

10

In specific embodiments it can be particularly preferred to select from pH-sensitive polymers, like, for example, however not exclusively: poly(acrylic acid) and derivatives, for example: homopolymers like poly(amino carboxylic acid), poly(acrylic acid), poly(methyl acrylic acid) and their copolymers. This applies likewise for polysaccharides like celluloseacetatephthalate, hydroxypropylmethylcellulosephthalate, hydroxypropylmethylcellulosesuccinate, celluloseacetatetrimellitate and chitosan.

15

In certain embodiments it can be especially preferred to select from temperature sensitive polymers, like for example, however not exclusively: poly(N-isopropylacrylamide-co-sodium-acrylate-co-n-N-alkylacrylamide), poly(N-methyl-N-n-propylacrylamide), poly(N-methyl-N-isopropylacrylamide), poly(N-N-propylmethacrylamide), poly(N-isopropylacrylamide), poly(N,N-diethylacrylamide), poly(N-isopropylmethacrylamide), poly(N-cyclopropylacrylamide), poly(N-ethylacrylamide), poly(N-ethylmethacrylamide), poly(N-methyl-N-ethylacrylamide), poly(N-cyclopropylacrylamide). Other polymers with thermogel characteristics are hydroxypropylcellulose, methylcellulose,

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hydroxypropylmethylcellulose, ethylhydroxyethylcellulose and pluronics like F-127, L-122, L-92, L-81, L-61.

- 5 In certain embodiments it can be especially preferred to use the polymers for the encapsulation of signal-generating agents, wherein predominantly no covalent bond between mono-, bi- tri- or multifunctional signal generating agents exists, or the signal-generating agents linked in the polymers as described above are provided in the form of polymer spheres or suspension or emulsion particles. The manufacture of
- 10 such capsules by the way of mini- or micro-emulsion is well known in the art. AU 9169501, EP 1205492, U.S. Patent 6,380,281, CN 1262692T, U.S. 2004192838, EP 1401878, EP 1352915, CA 1336218, EP 1240215, BE 949722, DE 10037656 provide an overview, further S. Kirsch, K. Landfester, O. Shaffer, M. S. El-Aasser: "Particle morphology of carboxylated poly-(n-butyl acrylate)/(poly(methyl
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MATERIALS/COMPONENTS

20

Implantable medical devices or materials for implantable medical devices or their components are one part of the combination in accordance with the invention.

- Fundamentally it has to be decided whether a bulk material with signal-generating properties is to be provided in which the signal generating agents are bound into the
- 25 material matrix of the implantable medical device, or whether the prepared medical device is to be provided, at least in part, with a signal-generating coating. In accordance with the invention, the possibility of combining both variants also exists.

In one generally applicable embodiment, the medical device itself is part of the inventive combination, and the device is combined with the at least one signal-generating agent and the at least one therapeutically active agent. This may be the incorporation of the signal-generating agent(s) and the therapeutically active agent(s) into the material of the implantable device itself, which is especially preferred if the device is made of resorbable or degradable materials.

In another generally applicable embodiment, the implantable device is itself not part of the inventive combination, and may be equipped, for example coated with a coating comprising the inventive combination, i.e. the at least one signal-generating device, the at least one therapeutically active agent and at least one material for the manufacture of an implantable medical device, which in this case may be a for example a suitable coating material like e.g. pyrolytic carbon, a polymer, a film coating or the like.

The term "at least one material for the preparation of an implantable medical device and/or at least one component of an implantable medical device" includes all the above described embodiments.

In accordance with the invention the implantable medical device or component of the implantable medical device provided can consist of a planar or spherical body, or any desired three-dimensional shape in different dimensions, also especially tubular or other hollow body shapes. The shape of the implantable medical device or component of the implantable medical device is not relevant to the application of the present invention.

With implantable medical devices any devices are designated which are incorporated into an organism as ultra short term, short term or long term devices for diagnostic, or therapeutic or prophylactic or combined diagnostic-therapeutic/prophylactic purposes. In the following the terms "implantable medical device" and "implant" are

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used synonymously. In accordance with the invention the selected organisms concern mammals. Mammals in accordance with the invention include all mammals, for example, however not exclusively, domestic animals like dogs and cats, agricultural livestock such as cattle, sheep, or goats, laboratory animals like mice, rats, primates
5 like apes, chimpanzees etc., and humans. In preferred embodiments implants and implanted active substances are to be selected which are designated for utilization inhuman.

The implantable medical devices to be chosen are not limited to any particular
10 implant type so that, for example, however not exclusively, one can elect from vessel endoprotheses, intraluminal endoprotheses, stents, coronary stents, peripheral stents, pacemakers or parts thereof, surgical and orthopedic implants for temporary purposes like joint socket inserts, surgical screws, plates, nails, implantable orthopedic supporting aids, surgical and orthopedic implants such as bones or joint prostheses,
15 for example artificial hip or knee joints bone and body vertebra means, artificial hearts or parts thereof, artificial heart valves, cardiac pacemakers housings, electrodes, subcutaneous and/or intramuscular implants, active substance repositories or microchips or the like. Materials for implantable medical devices can be selected from non-degradable or completely degradable materials or any combinations
20 thereof. Implant materials can moreover consist of entirely metal-based materials or alloys or composites, also laminated materials, carbon or carbon composites also composite materials of these, or any desired combinations of the named materials.

In certain embodiments ceramic and/or metal-based materials are especially
25 preferred, as for example amorphous and/or (partly) crystalline carbon, massive carbon material ("Vollkarbon"), porous carbon, graphite, carbon composite materials, carbon fibers, ceramics like e.g. zeolites, silicates, aluminum oxides, aluminum silicates, silicon carbide, silicon nitride; metal carbides, metal oxides,

metal nitrides, metal carbonitrides, metal oxycarbides, metal oxynitrides and metal oxycarbonitrides of the transition metals like titanium, zirconium, hafnium, vanadium, niobium, tantalum, chromium, molybdenum, tungsten, manganese, rhenium, iron, cobalt, nickel; metals and metal alloys, especially of the noble metals gold, silver, ruthenium, rhodium, palladium, osmium, iridium, platinum; metals and metal alloys of titanium, zirconium, hafnium, vanadium, niobium, tantalum, chromium, molybdenum, tungsten, manganese, rhenium, iron, cobalt, nickel, copper, magnesium; steel, especially stainless steel, especially Fe-18Cr-14Ni-2.5Mo ("316LVM" ASTM F138), Fe-21Cr-10Ni-3.5Mn-2.5Mo (ASTM F 1586), Fe-22Cr-13Ni-5Mn (ASTM F 1314), Fe-23Mn-21Cr-1Mo-1N (nickel-free stainless steel) or platinum containing radiopaque steel alloys, so called PERSS (platinum enhanced radiopaque stainless steel alloys), as well as shape memory alloys like nitinol, nickel-titanium alloy, glass, stone, glass fibers, minerals, natural or synthetic bone substance, imitation bone based on alkaline earth carbonates like calcium carbonate magnesium carbonate, strontium carbonate, hydroxyapatite as well as any combinations of the said materials.

In further embodiments polymers are preferred, for example chosen based on polyacrylates like polymethyl methacrylates or from unsaturated polyesters, from saturated polyesters, a polyolefin (for example polyethylene, polypropylene, polybutylene, and the like), an alkyd resin, an epoxy-polymer, a polyamide, a polyimide, polyetherimide, a polyamideimide, a polyesterimide, a polyesteramideimide, polyurethane, polycarbonate, polystyrene, polyphenol, polyvinylester, polysilicone, polyacetal, celluloseacetate, polyvinyl chloride, polyvinyl acetate, polyvinyl alcohols, polysulfones, polyphenylsulfones, polyethersulfones, polyketones, polyetherketones, polyetheretherketones, polyetherketoneketones, polybenzimidazoles, polybenzoxazoles, polybenzthiazoles,

polyfluorocarbons, polyphenyleneethers, polyarylates, cyanatoester-polymers, copolymers of two or more of those named and the like.

Useable polymers are especially acrylics so preferred are monoacrylates, diacrylates,
5 triacrylates, tetraacrylates, pentacrylates, and the like. Examples for polyacrylates are
polyisobornylacrylate, polyisobornylmethacrylates, polyethoxyethoxyethylacrylates,
poly-2-carboxyethylacrylates, polyethylhexylacrylates, poly-2-
hydroxyethylacrylates, poly-2-phenoxyethylacrylates, poly-2-
phenoxyethylmethacrylates, poly-2-ethylbutylmethacrylates, poly-9-
10 anthracenylmethyl methacrylates, poly-4-chlorophenylacrylates,
polycyclohexylacrylates, polydicyclopentenylacrylates, poly-2-(N,N-
diethylamino)ethylmethacrylates, poly-dimethylaminoethylacrylates, poly-
caprolactone 2-(methacryloxy)ethyl esters, or polyfurfurylmethacrylates,
poly(ethylene glycol)methacrylates, polyacrylic acid and poly(propylene
15 glycol)methacrylates.

Examples of preferred useable diacrylates, from which polyacrylates can be
manufactured are 2,2-bis(4-methacryloxyphenyl)propane, 1,2-butanedioldiacrylate,
1,4-butanedioldiacrylate, 1,4-butanedioldimethacrylate, 1,4-
20 cyclohexanedioldimethacrylate, 1,10-decanedioldimethacrylate,
diethyleneglycoldiacrylate, dipropyleneglycoldiacrylate,
dimethylpropanedioldimethacrylate, triethyleneglycoldimethacrylate,
tetraethyleneglycoldimethacrylate, 1,6-hexanedioldiacrylate,
neopentylglycoldiacrylate, polyethyleneglycoldimethacrylate,
25 tripropyleneglycoldiacrylate, 2,2-bis[4-(2-acryloxyethoxy)phenyl]propane, 2,2-bis[4-
(2-hydroxy-3-methacryloxypropoxy)phenyl]propane, bis(2-methacryloxyethyl)N,N-
1,9-nonylene-biscarbamate, 1,4-cyclohexanedioldimethacrylate, and diacrylic
urethane oligomers.

- Examples for triacrylates, which can be used for the manufacture of polyacrylates are preferred tris(2-hydroxyethyl)isocyanuratetrimethacrylate, tris(2-hydroxyethyl)isocyanuratetriacrylate, trimethylolpropanetrimethacrylate, trimethylolpropanetriacrylate or pentaerythritol-triacrylate. Examples for preferred tetraacrylate are pentaerythritoltetraacrylate, ditrimethylpropane tetraacrylate, or ethoxylated pentaerythritoltetraacrylate. Examples for pentaacrylates are dipentaerythritolpentaacrylate and pentaacrylate esters.
- 10 Polyacrylates also include other unsaturated aliphatic organic compounds such as for example polyacrylamides and unsaturated polyesters from condensation reactions of unsaturated dicarboxylic acids and diols, and vinyl compounds, but also compounds with terminal double bonds. Examples for vinyl compounds are N-vinylpyrrolidone, styrene, vinyl naphthalene or vinylphthalimide. To the especially preferred
- 15 methacrylamide derivatives belong N-alkyl- or N-alkylene-substituted or unsubstituted (meth)acryl amide, like for example acryl amide, methacrylamide, N-methacrylamide, N-methylmethacrylamide, N-ethylacrylamide, N,N-dimethylacrylamide, N,N-dimethylmethacrylamide, N,N-diethylacrylamide, N-ethylmethacrylamide, N-methyl-N-ethylacrylamide, N-isopropylacrylamide, N-n-propylacrylamide, N-isopropylmethacrylamide, N-n-propylmethacrylamide, N-acryloylpyrrolidine, N-methacryloylpyrrolidine, N-acryloylpiperidine, N-methacryloylpiperidine, N-acryloylhexahydroazepine, N-acryloylmorpholine or N-methacryloylmorpholine.
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- 25 Other useful polymers in accordance with the invention are unsaturated and saturated polyesters, especially also including alkyd resins. The polyesters can contain polymer chains, of a various number of saturated or aromatic dibasic acids and anhydrides. Further epoxy resins, which can be used monomers, oligomers or

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polymers which contain one or a plurality of oxiran rings, have an aliphatic, aromatic or mixed aliphatic-aromatic molecular structure, or exclusively non benzenoids, therefore aliphatic or cycloaliphatic, structures with or without substituents like halogens, ester groups, ether groups, sulfonate groups, siloxane groups, nitro groups or phosphate groups or any combinations thereof. Specially preferred are epoxy resins of the glycidyl-epoxy type, for example having diglycidylether groups of bisphenol-A. Especially preferred are further amino derivatized epoxy resins, for example tetraglycidyl-diaminodiphenylmethane, triglycidyl-p-aminophenol, triglycidyl-m-aminophenol or triglycidylaminocresol and their isomers, phenol derivatized epoxy resins like for example bisphenol-A epoxy resin, bisphenol-F epoxy resin, bisphenol-S epoxy-resins, phenol-novolak-epoxy resins, cresol-novolak-epoxy resins or resorcinol epoxy resins, or alicyclic epoxy resins. Furthermore halogenated epoxy resins, glycidylether of polyhydric phenols, diglycidylether of bisphenol A, glycidylethers of phenol-formaldehyde novolak resins and resorcinol-diglycidylether, as well as other epoxy resins as described in U.S. Patent 3,018,262 and incorporated herewith by reference explicitly. In accordance with the invention the selection is not restricted to the named epoxy resins alone, especially mixtures of two or three epoxy resins can also be chosen as well also as mono-epoxy components. The epoxy resins to be selected also include UV-cross-linked and cycloaliphatic resins.

Preferred polymers are also polyamides, like for example aliphatic or aromatic polyamides (Please include examples), inter alia also in specific embodiments Nylon-6-(polycaprolactam), nylon 6/6 (polyhexamethyleneadipamide), nylon 6/10, nylon 6/12, nylon 6/T (polyhexamethylene terephthalamide), nylon 7 (polyenanthamide), nylon 8 (polycapryllactam), nylon 9 (polypelargonamide), nylon 10, nylon 11, nylon 12, nylon 55, nylon XD6 (poly meta-xylylene adipamide), nylon 6/I, poly-alanine.

Further, however not restricted thereto, polymers which are preferably employed are polyimides, polyetherimides, polyamideimides, polyesterimides, polyesteramideimides.

5

In a specific embodiment conductive polymers are selected, preferably from saturated or unsaturated polyparaphenylenevinylene, polyparaphenylene, polyaniline, polythiophene, polyazines, polyfuranes, polypyrrole, polyselenophene, poly-p-phenylenesulfide, polyacetylene either as monomers, oligomers or polymers, in any combination and mixtures with other monomers, oligomers or polymers or copolymers of the monomers named above. Especially preferred contain one or a plurality of organic, for example alkyl or aryl radicals or the like, or inorganic radicals, such as for example silicon or germanium or the like, or any mixtures herefrom. Preferred are conducting or semi conducting polymers with resistivities between 10^{12} and 10^5 ohm cm. It can be especially preferred to choose such polymers in which complexed metal salts are contained which is why polymers are to be preferred which contain nitrogen, oxygen, sulfur or halides or unsaturated double bonds or triple bonds, and others which are suitable for complex formation. For example, without restricting the choice of suitable polymers thereto, elastomers like polyurethanes and rubbers, adhesive polymers and plastics are to be mentioned. Preferred metal salts include transition metal halides such as CuCl_2 , CuBr_2 , CoCl_2 , ZnCl_2 , NiCl_2 , FeCl_2 , FeBr_2 , FeBr_3 , CuI_2 , FeCl_3 , FeI_3 , or FeI_2 , furthermore salts like $\text{Cu}(\text{NO}_3)_2$, metal lactates, metal glutamates, metal succinates, metal tartrates, metal phosphates, metal oxalates, LiBF_4 , and $\text{H}_4\text{Fe}(\text{CN})_6$ and the like.

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In a specific embodiment conductive polymers are selected, preferably from saturated or unsaturated polyparaphenylenevinylene, polyparaphenylenes, polyanilines, polythiophenes, polyazines, polyfuranes, polypyrroles,

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polyselenophenes, poly-p-phenylenesulfides, polyacetylenes either as monomers, oligomers or polymers, in arbitrary combination and mixtures with other monomers, oligomers or polymers or copolymers of the monomers named above. Especially preferred contain one or a plurality of organic, for example alkyl or aryl radicals or
5 similar, or inorganic radicals, such as for example silicon or germanium or similar, or arbitrary mixtures here from. Preferred are conducting or semi conducting polymers with resistivities between 10^{12} and 10^5 ohm cm. It can be especially preferred to choose such polymers in which complexed metal salts are contained which is why polymers are to be preferred which contain nitrogen, oxygen, sulfur or halides or
10 unsaturated double bonds or triple bonds, and others which are suitable for complex formation. Here for example can be named without the choice of suitable polymers being restrictive, elastomers like polyurethane and rubber, adhesive polymers and plastics. Preferred metal salts include transition metal halides such as CuCl_2 , CuBr_2 , CoCl_2 , ZnCl_2 , NiCl_2 , FeCl_2 , FeBr_2 , FeBr_3 , CuI_2 , FeCl_3 , FeI_3 , or FeI_2 , furthermore
15 salts like $\text{Cu}(\text{NO}_3)_2$, metal lactates, metal glutamates, metal succinates, metal tartrates, metal phosphates, metal oxalates, LiBF_4 , and $\text{H}_4\text{Fe}(\text{CN})_6$ and similar .

Furthermore, especially biocompatible, here biodegradable polymers are preferred, for example, however not exclusively, collagens, albumin, gelatin, hyaluronic acid,
20 starch, cellulose (methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulose-phthalate; further casein, dextran, polysaccharides, fibrinogen, poly(D,L-lactide), poly(D,L-lactide-co-glycolide), poly(glycolide), poly(hydroxybutylate), poly(alkyl carbonate), poly(orthoester), polyesters, poly(hydroxyvaleric acid), polydioxanone,
25 poly(ethylene-terephthalate), poly(malic acid), poly(tartronic acid), polyanhydrides, polyphosphazenes, poly(amino acids), and all their co-polymers or any mixtures thereof.

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Within the degradable materials one may select especially preferably also metal-based materials such as for example biodegradable or biocorrosible metal alloys, like for example, but however not exclusively magnesium alloys, or degradable glass-ceramic materials like bioglass, silicates, or ceramic or ceramic type materials such as hydroxyapatite and the like.

Especially preferred implantable medical devices are for example, however not exclusively, non-degradable, or partly degradable or completely biodegradable devices selected from implant types for the complete or partial bone replacement, for the complete or partial joint replacement, for the complete or partial vessel replacement, coronary or peripheral stents, or other endoluminal vessel implants, for the complete or partial vessel replacement, active agent repositories or seed implants.

MATERIAL CHOICE

In accordance with the invention the choice of the individual elements of the present invention has a special importance. In the manufacture or use of signal-generating materials and the selection of the implants or implant materials provided, the provision of the signal generating agents of the implant type and implant purpose, according to the primary medical indication and the wanted signal generating modalities has to be considered. In accordance with the invention the fundamental teaching provides as follows:

Determination of the purpose of the signal generating agents, under which the following is to be established:

- a) whether the signal generating agents are to be selected exclusively for marking the implantable medical device;

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- b) whether the signal generating agents are to be selected exclusively for marking of surrounding tissue or of compartments at the immediate or communicable boundary area of the implantable medical device;
- 5 c) whether the signal-generating agents are to be chosen exclusively for marking of any desired tissues, cell types, organs or organ regions independent of the boundary area for the implantable medical devices, wherein such implantable medical devices can have the exclusive purpose of bringing signal-generating agents into the organism;
- 10 d) whether the signal-generating agents in addition to marking of the implant are also to be selected for marking the surrounding tissue or compartments in the immediate or communicable boundary area of the implant;
- e) whether the signal-generating agents, in addition to marking the implantable medical device, are also to be selected for marking any desired independent tissues, cell types, organs or organ regions of the boundary area of the
15 implantable medical device, wherein such devices can have the exclusive purpose to bring signal-generating agents into the organism;
- f) whether the signal generating agents are not or mainly not to be chosen for marking of the implantable medical devices, but mainly for the marking of surrounding tissues, or of compartments in the immediate or communicable
20 boundary area to the implantable medical device;
- g) whether the signal-generating agents are not to be chosen mainly or not at all for marking of the implantable medical devices but mainly for the marking the any tissues, cell types, organs or organ regions independently of their boundary area to the device, wherein such devices can have the exclusive
25 purpose of bringing signal-generating agents into the organism;
- h) whether the signal generating agents are not to be selected or mainly not for the marking of implantable medical devices, but in addition to the marking of surrounding tissues, or of compartments in the immediate or communicable

boundary area to the device also for marking of any cell types, organs or organ regions independent of the device boundary area, wherein such devices can have the exclusive purpose to bring signal-generating agents into the organism;

- 5 i) whether signal-generating agents are also to be chosen combined with therapeutic agents and should fulfill a through h);
- j) whether signal-generating agents are also to be chosen as combined signal-generating agents with different signal modalities, wherein under modalities are denoted the physical and chemical properties and the detection methods;
- 10 k) whether signal-generating agents selected so far under a) through j) are to be chosen from direct or indirect or mixed signal-generating agents;

Further, determination of the duration of detection of the signal-generating agents, under which it has to be determined especially:

- 15 a) whether signal-generating agents should be verifiable for ultra-short periods, in accordance with the definition for detection periods of a few seconds up to a maxim of 3 days;
- b) whether signal generating agents should be verifiable for short periods, in accordance with the definition from 3 days to 3 months.
- 20 c) whether signal-generating agents should be long term verifiable in accordance with the definition for 3 months and longer;
- d) whether signal generating agents be permanently verifiable for at least 12 months or longer, preferably over the total lifetime of a non-degradable
- 25 implant;

Further, determination of the preferred modalities, under which it has to be determined especially:

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- a) which modality is preferred corresponding to the detection method, for example radiographic methods for X-ray, MRI and fluorescence based methods;
- 5 b) which modalities should preferably be combined and made available, for example the combination of radiopaque and paramagnetic signal-generating agents;
- c) which modalities are to be chosen in combination with therapeutic signal-generating agents;
- 10 And finally the functionality of signal-generating agents in combination with the underlying implantable medical device, under it which especially to be determined:
 - a) whether signal-generating agents should be chosen exclusively for verification of the correct anatomical location;
 - 15 b) whether signal-generating agents are to be chosen for the control of the operation of the implantable medical device, for example, however not exclusively, for biodegradable implants to detect the course of the degradation;
 - c) whether for the signal-generating agents exclusively the interaction of the implantable medical devices with the bordering tissues should be detected, for example, however not exclusively, the engraftment and/or inflammatory reactions in the immediate or communicable surroundings of an implant;
 - 20 d) whether for signal-generating agents exclusively the release of additives should be controlled, especially for so-called combined implantable medical device with drug-delivery function, such as for example, however not
 - 25 exclusively, drug-eluting stents, here preferably through use of combined signal-generating agents combined with therapeutic agents;

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- e) whether signal-generating agents having at least one of the functions named under a) through d) together with at least one further or a plurality of the functions named under a) through d) should be fulfilled;

5 In accordance with the invention the underlying material or the composition or combination of implantable medical devices or of components of implantable medical devices, or the combination according to the invention is to be chosen from non-degradable or partially degradable or completely degradable materials. The choice of the composition or combination can follow the expect of the signal-
10 generating purpose and of the function of signal-generating agents, or conversely the signal-generating agents and their provided form as a function of the selected material of an implantable medical device. It is clear to the person skilled in the art that the material choice preferred has to be made according to the purpose of use and purpose of indication of the implantable medical device and the underlying primary
15 illness. Nevertheless there are the following criteria of choice for an improved implantable medical device in accordance with the invention:

The provision of implant materials for complete or partial introduction of signal-generating agents into the integrated material system, wherein it is to be established:

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- a) whether the material is manufactured by means of thermal sintering processes, wherein the integration of signal-generating material into the implant matrix is carried out before or during the manufacture;

b) whether the material is manufactured by means of thermal sintering
25 processes, wherein the integration of the signal-generating material into the implant matrix is carried out after the manufacture wherein at least one open-pore material layer must be present;

- 5 c) whether the material is manufactured by means of chemical processes without thermal stress, which lead to a degradation or partial degradation of signal-generating materials in the corresponding provided form; wherein the integration of signal-generating material into the implant matrix is carried out before or during the manufacture;
- 10 d) whether the material is manufactured by means of chemical processes without thermal stress, which lead to a degradation or partial degradation of signal-generating materials in the implant matrix wherein the integration of signal-generating materials into the implant matrix is carried out after the manufacture, wherein at least one open-pore material layer must be present;
- 15 e) whether one is dealing with a completely, partially or non-degradable material wherein a through d) or as desired combinations thereof are possible;

15 The provision of implants for complete or partial incorporation of signal-generating agents as a coating, wherein it has to be established:

- 20 f) whether the coating is manufactured by means of thermal sintering processes, plasma spraying, sputtering methods, etc, wherein the integration of signal-generating materials into the coating is carried out before or during the manufacture;
- g) whether the coating is manufactured by means of thermal sintering processes, plasma spraying, sputtering methods, etc, wherein the integration of signal-generating materials into the coating is carried out after the manufacture, wherein the coating can be closed or porous;
- 25 h) whether the coating is manufactured by means of chemical processes or thermally which leads to a degradation or partial degradation of signal-generating agents in the corresponding provided form, wherein the

integration of signal-generating material into the coating is carried out before or during the manufacture;

- i) whether the coating is manufactured by means of chemical processes without thermal treatment which leads to a degradation or partial degradation of signal-generating agents in the corresponding provided form, wherein the integration of signal-generating material into the coating is carried out after the manufacture;
- j) whether one deals with a completely, or partially or non-degradable material, wherein f) through i) or any desired combinations of them are possible.

10

For the choice of essentially non-porous and non-degradable implants the coating of the implant is preferred in accordance with the invention. The coating can be selected from degradable or non-degradable materials, wherein the incorporation of signal-generating agents and/or therapeutically active agents can be carried out during or after manufacture. The person skilled in the art can select from any desired coating method corresponding to the prior art. Thermal coating methods necessitate the choice of thermally stable signal-generating agents. Non-thermal methods like spray-coating, dip-coating etc allow one to choose from a multiplicity of possible sorts of preparation and their combinations as desired. If degradable coatings are chosen, then biodegradable types of coatings are especially preferred, for example formulated with polymers are formulated, either as mixtures, wherein the signal-generating agents are provided from solutions, suspensions, emulsions, dispersions, powders and similar, or as with signal-generating agents covalently linked polymer formulations. Mostly preferred are degradable coatings having bi-functional, tri-functional or multi-functional signal-generating agents, most preferably combined with at least one therapeutic agent.

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- In a further preferred embodiment the implantable device or part thereof, e.g. a coating on the device, comprises a porous material, into which, whether it is degradable or not, signal-generating agents are incorporated, e.g. as a reticulated network of particles. In this embodiment it is preferred to select at least one
- 5 therapeutically active agent that can be soaked into the matrix by techniques known in the art, e.g. by using appropriate drug solvents the device can be dipped into or sprayed with a therapeutically active agent containing solution with subsequent incorporation of the drug into the matrix.
- 10 In a specific embodiment signal-generating agents are provided in porous inorganic, organic or inorganic-organic coatings especially preferred from composite materials. Here for example, porous coatings, however not exclusively, can comprise ceramic or metal-based materials, wherein these can also be biodegradable, for example, however not exclusively, from hydroxylapatites or analogs or derivatives or similar,
- 15 or degradable bioglass species. It is preferred to integrate these inherently signal-generating materials with other signal-generating agents, either of the same modality for the strengthening of the image-forming signal, or one or a plurality of especially preferred other modalities; particularly signal-generating agents are chosen from nanoparticles. For degradable coatings it is preferred to choose biocompatible signal-
- 20 generating agents. Mostly it is preferred to provide porous coatings from signal-generating agents, for example but not exclusively, from non-degradable or degradable inorganic or organic or mixed inorganic-organic composites, with polymer provided forms, nano- or micro-morphous provided forms or from metal-based nanoparticles. Degradable implants are preferably provided with degradable
- 25 signal-generating coatings, preferably from degradable materials, which have the same or similar or shorter degradation times. The coating of non-porous degradable implants is in accordance with the invention particularly preferred, if the signal generation should mainly fulfill the purpose of verifying the correct anatomical

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location or is semi quantitatively involved in connection with the course and therapy control of the degradation, of engraftment and the interaction with the surrounding tissues, however not exclusively. Further, the coating of non-porous and degradable implants is especially preferred when its material leads to an impairment of the
5 implant function relative to the material properties if signal-generating agents are incorporated into the material composite. So for example with biodegradable implants like stents, which comprise biodegradable polymers such as PLA, mechanical stability for a functional implant function is not provided, if foreign substances like pharmacologically active substances are employed. Signal generating
10 coatings of degradable implants are especially preferred provided in the form of coatings, in which signal-generating agents in as desired forms, preferably as biocompatible nanoparticles, liposomes, micelles, micro spheres, etc. are embedded in degradable polymers. Especially preferred are coatings, which have radiopaque signaling properties, or contain bi-, tri-, or multifunctional signal-generating agents,
15 especially preferred with therapeutic agents.

In a specific embodiment, implants are prepared from biocompatible, essentially non-toxic metal alloys, which are degraded by means of corrosion, for example, however not exclusively, magnesium or zinc-based alloys. If from the materials
20 therapeutically active substances are released during the decomposition of these implants in the body, one can optionally, in accordance with the invention, do without the addition of a separate active ingredient.

Thus, in preferred embodiments, a magnesium or zinc alloy based implant or part of
25 an implant, e.g a stent, is used, which comprises in itself the therapeutically active agents, because in the human or animal organism, magnesium ions are liberated by and during degradation in body fluids, resulting in the physiologically induced formation of H₂, hydroxyl apatite and magnesium ions. In these embodiments, the

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release and availability of magnesium ions and the formation of hydroxyl apatite have biologic effects well known in the art.

Preferably, the implant or part thereof comprises the magnesium and/or zinc in the
5 implant construction material itself, or in a coating, for example by partially or fully
coating the implant with magnesium and/or zinc particles embedded in a polymeric
matrix or other coating material. In these embodiments the combination of
therapeutically active and signalling agent together with the implant material is
constituted by the use of the signalling agent, Mg or Zn as a component of the alloy
10 itself or as a part of the implant, or as a part of a coating.

It is further preferred to provide such implants with biodegradable signal-generating
coatings, especially preferred, although not exclusively, with signal-generating
agents, which are provided directly or incorporated into degradable polymers as
15 nanoparticles, in the form of liposomes, microspheres, macrospheres, encapsulated in
micelles or polymers, or bonded covalently to polymers, mostly preferred as bi-, tri-,
or multifunctional signal-generating agents, especially, however not exclusively,
together with at least one therapeutic agent. Further, it is preferred to provide such
implants with biodegradable porous coatings, for example of hydroxylapatite and
20 derivatives or analogs, or bioglass, wherein either biocompatible, preferably
biodegradable, signal-generating agents of nanomorphous particles are incorporated
into the porous coating, or any desired form of biocompatible or biodegradable
signal-generating agents, or both combined, into the hollow spaces of the porous
matrix. Also, mostly preferred, degradable porous coatings of nanomorphous
25 particles can be provided, which are selected from signal-generating agents, wherein
the hollow spaces of such signal generating porous coatings can be charged
additionally with signal-generating agents of any form. Further, non-porous

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degradable coatings of signal-generating agents are possible, preferably made of degradable nanomorphous particles.

- For the choice of essentially non-porous and essentially non-degradable implants, in accordance with the invention the signal-generating agents can be added as a part of the precursor components for the implant material. If thermal methods are selected for implant manufacture corresponding to the prior art, thermally stable forms of the signal-generating agents are preferred. For metal-based implant materials, signal-generating agents are preferred which impart the inherent signal-generating properties of the starting material used at least another additional signal-generating property from the modalities of the starting material. It is further preferred for the essentially non-porous and non-degradable implants from polymer materials or polymer composite materials to select from such signal-generating agents, which can be added to the reactant components formed from solutions, emulsions, suspensions, dispersions, powders etc. or as covalent components from monomers, dimers, trimers or oligomers or else prepolymeric precursors which can be synthesized to polymers, and to produce the material there from. For the essentially non-porous and non-degradable implants from polymeric, materials or polymer composite materials it is preferred to provide at least one modality representing signal-generating property, preferably bi-functional, tri-functional or multifunctional signal-generating agents, wherein non-porous and non-degradable materials or implants in accordance with the invention do not contain any therapeutic agents or targeting groups within the material composite.
- It can be especially preferred for non-porous and non-degradable implants to provide the reactant components of the implant material with signal-generating agents in a suitably finished form and to provide the finished implant with an additional signal-generating coating.

For the choice of essentially non-porous and essentially degradable implants, in accordance with the invention the signal-generating agents can be added as a part of the precursor components to the implant material. Preferred implant materials are

5 polymers or polymer composites as well as degradable metal-based materials or their degradable composites or materials based on naturally occurring apatites, hydroxylapatites, their analogs and derivatives, or materials comparable to bone substitute or based on bioglass species. It is further preferred for essentially non-porous and essentially degradable implants from polymer materials or polymer

10 composite materials to select such signal-generating agents which can be added to the reactant components from solutions, emulsions, suspensions, dispersions, powders etc. or as covalent components of monomers, dimers, trimers or oligomers or other pre-polymer precursors, which can be synthesized to polymers and to produce the active substance there from. In contrast to WO04/064611 signal-

15 generating agents are added to biodegradable polymers like those or polylactides, polyglycolides, their derivatives and mixtures thereof or their copolymers, which have preferably radiopaque properties and combined have at least one other modality, or at least bifunctional radiopaque properties in combination with a therapeutic agent or at least one non-radiopaque modality. Especially preferred are

20 those signal-generating agents which are coupled with one or a plurality of targeting groups and/or a plurality of therapeutic agents. This is further preferred for materials which are based on naturally occurring apatites, hydroxyl apatites, their analogs and derivatives, comparable bone substitutes or bioglass and the like. It can be especially preferred for non-porous and degradable implants to add signal-generating agents to

25 the reactant components of the implant in a suitable form and to provide the shaped implant with an additional signal-generating coating.

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In a specific embodiment, implants are prepared from biocompatible, essentially non-toxic, metal alloys, which are degraded by means of corrosion, for example, however not exclusively, magnesium- or zinc-based alloys. It is preferred to add thermally stable finished forms of signal-generating agents to the educt components of such implant materials to if these are manufactured in thermal methods corresponding to the prior art. Signal-generating agents are especially preferred which have radiopaque properties, bi- and tri-functional as well as multifunctional signal-generating agents in suitable provided forms, mostly preferred signal-generating agents coupled with therapeutic agents and/or targeting groups.

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It can be especially preferred for nonporous and degradable materials that signal-generating agents are added to the reactant components of the implant materials in a suitable form, so as to provide the formed implant with an additional signal-generating coating.

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Porous, essentially non-degradable or degradable implants can already contain signal-generating agents in their material composite structure, for example as described above in accordance with the invention. It is especially preferred to provide porous implants with signal-generating agent after their manufacture. In

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accordance with the invention it is to be distinguished whether the implants in accordance with the manufacturing process already have a porous composite material, or whether implants are provided with porous coatings. Preferred, however not exclusively preferred are implants which have a porous material structure.

Preferred pore sizes in accordance with the invention are pores having a medium size from 1 nm to 10 μm , especially preferred 1 nm to 10 μm , mostly preferred 2 nm to 1 μm . The provision of at least one sufficiently porous surface is important, which can be loaded with signal-generating agents, Respective of the fact, whether this surface

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is created later, or not, whether the porosity is produced by a specific implant manufacturing process or whether it involves an open-pore material composite.

5 The signal generating agents are introduced into the porous compartment preferably from solutions, suspensions, dispersions or emulsions or with additives selected by to the person skilled in the art such as, surfactants, stabilizers, flow improvers etc. by means of suitable methods, for example, dipping, spraying, injection methods and other suitable prior art methods.

10 Porous implants can be selected from any materials like for example, however not exclusively, polymers, glass, metals, alloys, bones, stone, ceramics, minerals or composites. It is unimportant whether these are degradable, non-degradable or partially degradable. It is preferred to provide monofunctional signal-generating agents, it is mostly preferred to select bi-functional or tri-functional signal generating
15 agents especially preferred those coupled with therapeutic agents.

In another specific embodiment, porous materials are produced by introduction of appropriate forms of signal generating agents. Thus non-degradable polymers, polymer composites or ceramics or ceramic composites or metal-based materials or
20 metal-based composites or similar materials can already contain signal-generating materials in the form of fillers during the manufacturing process, so that they serve as components of the basic material matrix of the overall composition. It is then especially preferred to select signal-generating agents encapsulated in polymers, for example in the form of polymer capsules, drops or beads, produced by the way of
25 mini- or micro-emulsion, or especially for polymer-based materials to choose from signal-generating agents encapsulated in polymers, micelles, liposomes or micro spheres, or, however, from nanoparticles. Such implantable medical devices have a porous matrix structure in-vivo, when by dehydration or degradation and release of

the fillers and the signal-generating agents and/or therapeutic agents contained in the fillers, the basic material matrix remains.

In accordance with the invention, adjuvants or fillers are added to the composition or
5 combination of material. Adjuvants or filler materials can be chosen in order to allow
a bonding between the signal-generating agents or the therapeutic agents with the
implant material and/or between the agents. A further object of the adjuvants and
fillers can comprise to make possible the material bond of the composition or
combination by physical or chemical ways, or to modulate the elasto-mechanical,
10 chemical or biological properties. In particular the adjuvants and fillers are chosen as
described above in order to form micelles, micro spheres, macro spheres, or
liposomes, nano- micro- and macro-capsules, micro bubbles etc or functional units,
for example by attachment of appropriate functional groups and compounds. Further
the adjuvants and fillers are chosen, in order to attach the composition as a
15 component of an implantable medical device to another component or a part of the
implantable device, for example in the form of a coating.

Thus, the adjuvants can be comprised of polymer, non-polymer, organic or inorganic
or composite materials. The setting of the elasto-mechanical properties can be carried
20 out by adding carbon-, polymer- glass- or other fibers of any size in woven or non-
woven form.

It is especially preferred to chose adjuvants, which modulate, for example retard the
release of signal generating and/or therapeutic agents. The person skilled in the art,
25 referring to this, will choose the adjuvants that according to the purpose and location
of insertion of the implantable medical device, a degradable or non-degradable
material is chosen as component of the composition or combination, or a

hydrophobic or hydrophilic material or any desired mixtures thereof, or forms of crystalline, semi-crystalline or amorphous forms of such adjuvants.

For the release from partially degradable or degradable or non-degradable devices
5 the degradation rate in the physiological medium can be adjusted for example by the mixing of hydrophobic and hydrophilic adjuvants. Further, by the choice of substances by their melting point, the predominant presence of crystalline, semi-crystalline or amorphous phases, also of mixtures of hydrophobic and hydrophilic substances, can be adjusted, for example by selecting polymers having melting
10 points, close to, above or below the body temperature of the target organism, and so the solubility of the adjuvants, which for example exist as matrix, micelles, microspheres, liposomes or capsules or similar structures, and therewith set in the elution or erosion or degradation of the agents or the medical devices. Another possibility in accordance with the invention is to adjust the solids content of the adjuvants, and to
15 influence the desired leaching out, release or degradation rates therewith for example via the adjustment of coating thicknesses or matrix volumes.

In exemplary embodiments of the devices and methods of the present invention, an implantable medical device, e.g. a metallic stent or a pacemaker electrode or an
20 artificial heart valve, is coated with a porous coating, for example with a pyrolytic carbon coating as described in DE 202004009060U. The coating is subsequently loaded with at least one signal-generating agent as described above, and simultaneously or subsequently with at least one therapeutic agent as described above, selected in accordance with the intended use of the device, wherein the order
25 of the loading with the different agents may be selected as deemed suitably. The loading may be done by spraying, impregnating with solutions or in any other suitable way. If necessary, further adjuvants or overcoatings may be applied, in order to control the release rates of the agents. The average release rates of the

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signal-generating agent and the therapeutic agent from the so produced implantable device may be determined by commonly used in-vitro tests in balanced salt solution or any other suitable media. From concentration measurements, optionally combined with non-invasive physical detection methods for the signal-generating agents, a correlation coefficient for the amount of therapeutic agent released per amount of signal intensity obtained from the signal-generating agent can be determined, which allows for an indirect determination of the amount of therapeutic agent released in relation to the signal intensity obtained from detecting the signal-generating agent. With this method, monitoring of the amount and the regional distribution of released therapeutic agent is accurately possible by simple, non-invasive physical detection methods.

DESCRIPTION OF THE FIGURES

Figure 1 shows the correlation between the release of paclitaxel from a coronary stent in the form of encapsulated nanoparticle adsorbed active substance and the *in-vivo* activity of the fluorescence color of the signal-generating agent Calcein-AM in accordance with a preferred embodiment of the invention.

The invention is now further explained in the following, based on examples, in order to represent the principle of the composition or combination and exemplary preferred embodiments, which do not indicate any necessary limitations to the invention as described in the paragraphs:

EXAMPLES

Example 1

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A commercially available, X-ray dense, non-fluorescing coronary stent from Fortimedix Company (KAON Stent), Netherlands, 18.5 mm long, and made of stainless steel 316L was coated with a coating of Carbon-Si composite material in accordance with DE 202004009060U. As precursor polymer a phenoxy resin was
5 used, Beckopox EP 401, from UCB Company, and a dispersion of commercially available Aerosil R972 from Degussa in methylethylketone was prepared. The solids content of the polymer amounted to 0.75 wt%, the solids content of Aerosil 0.25 wt%, the solids content of solvent 99 wt%. The precursor solution was sprayed onto the substrate as a polymer film, tempered by application of hot air at 350 °C in
10 ambient air and subsequently the crude weight of the polymer film was determined, wherein the coating had a surface area weight of about 2.53 g/m². The sample was subsequently examined in a Nikon fluorescence microscope for its inherent fluorescence. The crude coating did not have any fluorescence. Subsequently the sample was treated thermally in accordance with DE 202004009060U in a
15 commercial tube reactor. The thermal treatment was carried out under nitrogen atmosphere with a heat-up and cool-down ramp of 1.3 K/min with a holding temperature of 300 °C and a holding period time of 30 minutes. Subsequently the sample was treated in an ultrasonic bath in 10 ml of a 50 % ethanol solution at 30 °C for 20 minutes, washed and dried in a commercial convection oven at 90 °C. The
20 gravimetric analysis indicated a shrinkage after the thermal treatment of about 29 % and a surface area weight of the composite layer of glassy, amorphous carbon/Si of 1.81 g/m². The scanning electron microscope investigation shows a porous layer with average pore diameters of about 100nm. A subsequent investigation in a fluorescence microscope showed an intensive fluorescence of the coated coronary stent in the
25 green and blue region as well as a weak fluorescence in the red region.

Example 2

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As in Example 1, a commercially available, X-ray dense, non-fluorescing coronary stent from Fortimedix Company (KAON Stent), Netherlands, 18.5 mm long and made of 316L stainless steel was coated with a coating of Carbon-Si composite material in accordance with DE 202004009060U. For modification of the

5 fluorescence emission spectrum in the red region the composition of the precursor was modified. As the precursor polymer a phenoxy resin was used, Beckopox EP 401 from UCB Company, and combined with a dispersion of commercially available Aerosil R972 from Degussa, in methylethylketone. Additionally, a cross-linking agent was introduced, isophorone diisocyanate, from Sigma Aldrich Company. The

10 solids content of the polymer amounted to 0.55 wt%, the solids content of Aerosil 0.25 wt%, the solids content of the cross-linking agent 0.2 wt%, the solid portion of solvent 99 wt%. The precursor solution was sprayed onto the substrate as a polymer film, tempered by application of hot air at 350 °C in ambient air and subsequently the crude weight of the polymer film determined, wherein the coating had a surface area

15 weight of about 2.20 g/m². The sample was subsequently examined in a Nikon fluorescence microscope for its inherent fluorescence. The crude coating did not have any fluorescence. Subsequently the sample was treated thermally in accordance with DE 202004009060U in a commercial tube reactor. The thermal treatment was carried out under nitrogen atmosphere with a heat-up and cool-down ramp of 1.3

20 K/min with a holding temperature of 300 °C and a holding period of 30 minutes. Subsequently the sample was treated in an ultrasonic bath in 10 ml of a 50 % ethanol solution at 30°C for 20 minutes, washed and dried in a commercial convection oven at 90 °C. The gravimetric analysis indicated a shrinkage after the thermal treatment of about 23 % and a surface area weight of the composite layer of glass-like

25 amorphous carbon/Si of 1.69 g/m². The scanning electron microscope investigation shows a porous layer with average pore diameters of about 100 nm. A subsequent investigation in a fluorescence microscope showed an intensive fluorescence of the

coated coronary stent in the green and blue region as well as a strong fluorescence in the red region.

Example 3

- 5 The coronary stents produced in Example 1 and Example 2 were subsequently changed up with an active agent. Paclitaxel obtained from Sigma Aldrich was used as model substance. A Paclitaxel solution having a concentration of 43 g/l was prepared in ethanol. Before and after being changed by dipping in 5 ml of the ethanolic paclitaxel solution the samples were subjected to a gravimetric analysis..
- 10 The charge was carried out by means of dipping for 10 minutes in the active agent solution. The overall charge was determined from the increase in mass. The sample from Example 1 had a loading of 0.766 g/m^2 , the sample from Example a loading of 0.727 g/m^2 . After drying in air for 60 minutes another fluorescence microscopy investigation was carried out, which showed the same fluorescence characteristics as
- 15 for the unloaded porous coatings (strong blue and green fluorescence, sample from Example 1 weak red fluorescence, sample from Example 2 strong red fluorescence).

Example 4

- Three commercially available X-ray dense, non-fluorescing coronary stents from
- 20 Fortimedix Company (KAON Stent), Netherlands, 18.5 mm long, made of 316L stainless steel were coated with a coating of carbon-carbon composite material in accordance with DE 202004009060U. As precursor polymer a phenoxy resin was used, Beckopox EP 401 from UCB Company and from that a dispersion was prepared in methylethylketone with commercially available carbon black, Printex
- 25 alpha from Degussa and a fullerene mixture of C60 and C70 from FCC Company sold as Nanom-Mix. The solids content of the polymer amounted to 0.5 wt%, the solids content of carbon black 0.3 wt%, the solids content of fullerene mix 0.2 wt%, that of the solvent 99 wt%. The precursor solution was sprayed onto the substrate as

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a polymer film, tempered by application of hot air at 350 °C in ambient air and subsequently the crude weight of the polymer film determined, wherein the coating had a surface area weight of about 2.5 g/m². The sample was subsequently examined in a Nikon fluorescence microscope for its inherent fluorescence. The crude coating
5 did not have any fluorescence. Subsequently the sample was treated thermally in accordance with DE 202004009060U in a commercial tube reactor. The thermal treatment was carried out under nitrogen atmosphere with a heat-up and cool-down ramp of 1.3 K/min with a holding temperature of 300 °C and a holding period of 30 minutes. Subsequently the sample was treated in an ultrasonic bath in 10 ml of a 50
10 % ethanol solution at 30 °C for 20 minutes, washed and dried in a commercial convection oven at 90 °C. The gravimetric analysis indicated a shrinkage after the thermal treatment of about 30 % and a surface area weight of the composite coating of a glass-like amorphous carbon/pyrolytic carbon of 1.75 g/m². The scanning electron microscope revealed an average porosity of 1 μm. The fluorescence
15 microscopic investigation indicated no fluorescence of the coating.

For the active agent loading, firstly a 1 mM Calcein-AM-solution in DMSO from Mobitec Company, was diluted to 1:1000 in acetone and 0.5 mg of the calcein solution together with 20 mg poly(DL-lactide coglycolide) and 2 mg Paclitaxel in 3
20 ml of acetone were mixed in. The resulting solution was added with a constant flow rate of 10 ml/min to a solution of 0.1 % Poloxamer 188 (pluronic F68) in 0.05 M PBS buffer while stirring at 400 rpm, and the colloidal suspension stirred further for 3 h under light vacuum for evaporation of the solvents and subsequently completely dried for 14 h under full vacuum. The nanoparticles obtained with encapsulated
25 Paclitaxel and *in-vivo* fluorescence marker were subsequently re-suspended in ethanol and by determination of the solids content the concentration of the particle containing solution obtained.

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The three coated coronary stents were subsequently loaded with the particles by dipping and the charged weight determined gravimetrically. The average loading of the convection oven dried coronary stents amounted to $0.5 \text{ g/m}^2 \pm 0.05$. Subsequently the expanded stents were brought into 6 well-plates and incubated with about 10^5 cells/ml three times passaged COS-7 cell cultures (37.5°C , 5 % CO_2) in DMEM medium in a culture volume of 5 ml. Immediately after the expansion after 1, 3, 6, 12, 24, 36 as well as 2, 3, 4, 5, 7, 9, 12, 15, 21 and 30 days in each case the culture volumes were obtained, the released amounts determined by means of HPLC, and the medium in each case replaced. Further, the samples were investigated in the fluorescence microscope and the adherent cells investigated for fluorescence in the green region. By means of Lucia software from Nikon Company in each case an area of $0.5 \mu\text{m}^2$ was determined by means of the densitometric measurement of the average color intensity of the fluorescence intensity. The densitometric maximum was observed after 30 days and the intensity of the fluorescence values correlated to the release of calcein-AM in percentile versus time was determined.

The graph of Figure 1 collects the measured values and shows the correlation between the release of adsorbed Paclitaxel of the encapsulated nanoparticles from the coronary stent and the in-vivo activity of the fluorescent coloring of Calcein-AM. After a period of 35 days the samples were transferred into new culture vessels and incubated with fresh cell suspension. Paclitaxel could neither be identified in the medium nor was there any fluorescence coloring of the cell culture.

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the below claims is not to be limited to particular details set forth in the above description as many apparent variations

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thereof are possible without departing from the spirit or scope of the present invention.

Claims:

1. An implantable medical device or component thereof, comprising a combination comprising:
 - a) at least one signal generating agent, which in a physical, chemical and/or biological measurement or verification method leads to detectable signals,
 - b) at least one material for manufacture of an implantable medical device and/or at least one component of an implantable medical device,
 - c) at least one therapeutically active agent, which in an animal or human organism fulfills directly or indirectly a therapeutic function,
 - d) an adjuvant which allows to control the release of the at least one therapeutically active agent and the at least one signal-generating agent when the device is exposed to physiologic fluids and/or has been implanted into a human or animal organism.
2. The device or component according to claim 1, wherein the therapeutically active agent can be directly or indirectly released in an animal or human organism from the implantable medical device or a component of the implantable medical device.
3. The device or component according to claim 1, wherein the signal-generating agent in addition to its signal-generating function has at least a second function.
4. The device or component according to claim 1, wherein the signal-generating agent possesses signal-generating properties without a physical or chemical or biological stimulus or a physiologically conditioned *in-vivo* change.
5. The device or component according to claim 1, wherein the signal-generating agent gains its signal-generating properties through a physical, chemical or biological stimulus.

6. The device or component according to claim 1, wherein the signal-generating agent gains its a signal-generating properties through a physical, chemical or biological or, physiologically conditioned change *in-vivo*.

7. The device or component according to claim 1, wherein the material for the manufacture of an implantable medical device comprises biologically degradable materials.

8. The device or component according to claim 1, wherein the material for the manufacture of an implantable medical device comprises biologically non-degradable materials.

9. The device or component according to claim 1, wherein the material for the manufacture of an implantable medical device comprises a combination of biologically non-degradable materials and biologically degradable materials.

10. The device or component according to claim 3, wherein the second function or other functions is/are that of at least one therapeutically active agent.

11. The device or component according to claim 3, wherein the second function or other functions comprises that of at least one targeting group.

12. The device or component according to claim 3, wherein in addition to the signal-generating function at least the function of one therapeutically active agent and the function of at least one targeting group is present in the device or component.

13. The device or component according to one of the preceding claims, wherein the signal-generating agent comprises a first and at least one second unit,

which are covalently bonded to each other, wherein the first unit has a signal-generating function and the second unit or further units have other functions.

14. The device or component according to one of the preceding claims, wherein the signal-generating agent comprises a first and at least one second unit, which are bonded to each other non-covalently, wherein the first unit has a signal-generating function and the second unit or further units have other functions.

15. The device or component according to claim 13 or 14, wherein the function of the second unit or further units is that of at least one therapeutically active agent.

16. The device or component according to claim 13 or 14, wherein the function of the second unit or further units is that of at least one targeting group.

17. The device or component according to claim 13 or 14, wherein the function of the second unit and of at least one further unit is that of a therapeutically active agent and that of at least one targeting group.

18. The device or component according to one of the preceding claims, wherein the combination comprises a second signal-generating agent, wherein the second signal-generating agent is detectable with a measurement or verification method, with which the first signal-generating agent is essentially not detectable.

19. The device or component according to one of the preceding claims, wherein the implantable medical device comprises at least a region which shows a concentration gradient in the local distribution of the at least one signal-generating agent.

20. The device or component according to one of the preceding claims, wherein the implantable medical device comprises a first and a second coating layer,

wherein the concentration of the at least one signal-generating agent in the first layer differs from the concentration in the second coating layer.

21. The device or component according to one of the preceding claims, wherein the adjuvant(s) are biodegradable.

22. The device or component according to one of claims 1 to 20, wherein the adjuvant(s) are non-degradable.

23. The device or component according to one of claims 1 to 20, wherein the adjuvant(s) are partially biodegradable.

24. The device or component according to one of claims 1 to 20, wherein the adjuvant(s) are partly biodegradable or comprise mixtures of non-degradable, degradable and/or partly degradable materials.

25. The device or component according to one of the preceding claims, wherein the adjuvant is a retarding agent.

26. The device or component for the manufacture of implantable medical devices according to any one of the previous claims, comprising a first and a second signal-generating agent, which lead directly or indirectly to detectable signals in a physical, chemical and/or biological measurement or verification method, wherein the first agent in a method, in which the second agent leads to detectable signals is essentially not detectable.

27. The device or component according to claim 26, wherein the first signal-generating agent leads to detectable signals in methods including one of conventional X-ray methods, X-ray-based split-image methods like computer tomography, neutron transmission tomography, radio frequent magnetization including magnetic resonance tomography, methods based on radio nuclides including scintigraphy, single photon emission computed

tomography (SPECT), positron emission computed tomography (PET), ultrasonic-based methods, fluoroscopic methods, luminescence or fluorescence-based methods, including intravascular fluorescence spectroscopy, Raman spectroscopy, fluorescence emission spectroscopy, electrical impedance spectroscopy, colorimetry, optical coherence tomography, electron spin resonance (ESR), radiofrequency (RF), or a microwave laser method.

28. The device or component according to claim 26 or 27, wherein the second signal-generating agent leads to detectable signals in methods including at least one of conventional X-ray methods, X-ray based split-image methods including computer tomography, neutron-transmission tomography, radio frequency magnetization including magnetic resonance tomography, methods based on radio nuclides including scintigraphy, single photon emission computed tomography (SPECT), positron emission computed tomography (PET), ultrasonic based methods, fluoroscopic methods, luminescence, fluorescence based methods including intravascular fluorescence spectroscopy, Raman spectroscopy, fluorescence emission spectroscopy, electrical impedance spectroscopy, colorimetry, optical coherence tomography, electron spin resonance (ESR), radiofrequency (RF), or a microwave laser .

29. The device or component according to one of claims 26 through 28, wherein at least one of the first or second signal generating agent is selected from the group consisting of metals, metal oxides, metal carbides, metal nitrides, metal oxynitrides, metal carbonitrides, metal oxycarbides, metal oxynitrides, metal oxycarbonitrides, metal hydrides, metal alkoxides, metal halides, inorganic or organic metal salts, including salts and chelates from the lanthanide group with atomic numbers 57-83 or from the transition metals with atomic numbers 21-29, 42 or 44, metal polymers, metallocenes, and other organometallic compounds, including metal complexes with phthalocyanines.

30. The device or component according to one of the claims 26 through 28, wherein at least one of the first or second signal-generating agent is selected from the group consisting of magnetic materials or compounds, including those with paramagnetic, diamagnetic, super paramagnetic, ferrimagnetic or ferromagnetic properties, semiconducting

materials including those from the Groups II-VI, Groups III-V, or Group IV having absorption properties for radiation in the wavelength ranges from gamma rays up to microwave radiation and/or the property of emitting radiation.

31. The device or component according to one of claims 26 through 28, wherein at least one of the first or second signal-generating agent is selected from the group consisting of ionic and non-ionic halogenated agents, including 3-acetylamino-2,4,6-triiodobenzoic acid, 3,5-diacetamido-2,4,6-triiodobenzoic acid, 2,4,6-triiodo-3,5-dipropionamidobenzoic acid, 3-acetyl amino-5-((acetyl amino)methyl)-2,4,6-triiodobenzoic acid, 3-acetyl amino-5-((acetylmethylamino)-2,4,6-triiodobenzoic acid, 5-acetamido-2,4,6-triiodo-N-((methylcarbamoyl)methyl)isophthalamic acid, 5-(2-methoxyacetamido)-2,4,6-triiodo-N-[2-hydroxy-1-(methylcarbamoyl)-ethyl]-isophthalamic acid, 5-acetamido-2,4,6-triiodo-N-methylisophthalamic acid, 5-acetamido-2,4,6-triiodo-N-(2-hydroxyethyl)isophthalamic acid, 2-[[2,4,6-triiodo-3[(1-oxobutyl)amino]phenyl]methyl]butanoic acid, beta-(3-amino-2,4,6-triiodophenyl)-alpha-ethylpropionic acid, iopamidol, iotrolan, iodecimol, iodixanol, iogluco, ioglucomide, iogulamide, iomeprol or iopentol.

32. The device or component according to one of claims 26 through 28, wherein at least one of the first or second signal-generating agent is selected from the group consisting of carbon species, including carbides, fullerenes, fullerene-metal complexes, or endohedral fullerenes, which contain rare earths including cerium, neodymium, samarium, europium, gadolinium, terbium, dysprosium or holmium, or halogenated fullerenes.

33. The device or component according to one of claims 26 through 28, wherein the first or second signal-generating agent is selected from the group consisting of anionic and/or cationic lipids including halogenated anionic or cationic lipids.

34. The device or component according to one of claims 26 through 28, wherein the first or second signal-generating agent is selected from the group consisting of gases or *in-vivo* gas-forming substances including air, nitrogen, hydrogen, alkanes, or halogenated hydrocarbon gases including methyl chloride, perfluoroacetone, or perfluorobutane ,

optionally included in microbubbles or microspheres.

35. The device or component according to one of claims 26 through 28, wherein at least one of the first or second signal-generating agent is selected from the group consisting of recombinant or non-recombinant nucleic acids, proteins, peptides or polypeptides, including those which directly or indirectly induce the *in-vivo* formation or enrichment of signal-generating agents, including nucleic acids, which contain coding sequences for the expression of signal-generating agents, including metallo-protein complexes, dicarboxylate proteins, lactoferrin or ferritin, or those that regulate enrichment and/or homeostasis of physiologically available signal-generating agents including the iron regulatory protein (IRP), transferrin receptor, erythroid 5-aminolevulinate synthase.

36. The device or component according to one of claims 29 through 35, wherein at least one of the first or second signal-generating agent is provided in the form of polymeric and/or non-polymeric nano particles or micro particles, having an average size of 2 nm to 20 μm , or from 2 nm to 5 μm .

37. The device or component according to one of claims 29 through 36, wherein at least one of the first or second signal-generating agent is provided in the form of microspheres, macrospheres, micelles or liposomes, or encapsulated in polymeric shells.

38. The device or component according to one of claims 29 through 35, wherein at least one of the first or second signal-generating agent is provided in the form of biological vectors, for example transfection vectors such as virus particles or viruses, preferably adeno viruses, adeno virus associated viruses, herpes simplex viruses, retroviruses, alpha viruses, pox viruses, arena-viruses, vaccinia viruses, influenza viruses or polio viruses.

39. The device or component according to one of claims 29 through 35, wherein at least one of the first or second signal-generating agent is selected in the form of signal-generating agents or vectors containing cells, cell cultures, organized cell cultures, tissues, organs of any desired species, and non-human organisms which contain recombinant nucleic

acids with coding sequences for signal-generating agents.

40. The device or component according to one of claims 29 through 39, wherein at least one of the first or second signal-generating agent is provided in the form of a solution, a suspension, an emulsion, a dispersion or a solid material, or any mixtures thereof.

41. The device or component according to one of claims 26 through 28, wherein the first signal-generating agent is bonded covalently to the second signal-generating agent.

42. The device or component according to one of claims 26 through 28, wherein the first signal-generating agent is bonded non-covalently to the second signal-generating agent.

43. The device or component according to any one of the preceding claims, wherein the implantable medical device or component thereof is formed from a material which in at least one of the image forming methods applied in medical technology is not representable.

44. The device or component according to claim 43, wherein the material comprises at least one polymer, selected from the group consisting of polyurethane, collagens, albumin, gelatin, hyaluronic acid, starch, cellulose including methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulosephthalat, casein, dextrane, polysaccharides, fibrinogen, poly(D,L-lactide), poly(D,L-lactide-co-glycolide), poly(glycolides), poly(hydroxybutylate), poly(alkyl carbonates), poly(orthoesters), polyesters, poly(hydroxyvaleric acid), polydioxanone, poly(ethylene terephthalate), poly(malic acid), poly(tartronic acid), polyanhydrides, polyphosphohazenes, poly(amino acids), or copolymers thereof .

45. The device or component according to claim 43, wherein the material

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comprises at least one non-polymeric material selected from the group consisting of ceramic, glass, metals, alloys, bone, stone or minerals.

46. The device or component according to claim 43, wherein the material comprises a mixture of non-polymeric and polymeric materials.

47. The device or component according to claim 43, wherein the material comprises an organic, inorganic or mixed inorganic-organic composite material.

48. An implantable medical device or component thereof according to any one of the preceding claims, comprising magnesium and/or zinc.

49. The device of claim 48, being a stent.

50. The device of claim 49, wherein the stent is coated at least partially with a coating comprising magnesium and/or zinc particles, or alloy particles comprising magnesium and/or zinc.

51. The device of claim 49, wherein the stent or a part thereof is made of a material comprising magnesium and/or zinc or an alloy of any of these metals.

52. The device of any one of the preceding claims, comprising signal-generating agents in a porous reticulated network which can be loaded with therapeutically active agents.

53. The device of any one of the preceding claims, wherein the combination is in the form of a coating.

54. A method for the determination of the extent of the release of an active agent from an implantable medical device or component thereof as defined in any one of claims 1 through 53 comprising:

providing a completely or partially degradable implantable medical device or a completely or partially degradable component thereof, the device comprising at least one signal-generating agent, which leads directly or indirectly to detectable signals in a physical, chemical and/or biological measurement or verification method including an imaging method, and at least one therapeutically active agent to be released in a human or animal organism, wherein the device at least partially releases the therapeutically active agent(s) together with the signal generating agent(s) upon degradation of the device after insertion of the device into a human or animal organism:

determining the extent of therapeutically active agent(s) release by detecting the released signal-generating agent(s) with the use of non-invasive imaging methods.

55. A method for the determination of the extent of the release of an active agent from an implantable medical device or component thereof as defined in any one of claims 1 through 53, comprising:

providing a non-degradable implantable medical device or a component thereof the device comprising at least one signal-generating agent, which leads directly or indirectly to detectable signals in a physical, chemical and/or biological measurement or verification method, especially in an imaging method, and at least one therapeutically active agent to be released in a human or animal organism, wherein the device at least partially releases the therapeutically active agent(s) together with the signal generating agent(s) after insertion of the device into a human or animal organism;

determining the extent of therapeutically active agent(s) release by detecting the released signal-generating agent(s) with the use of non-invasive imaging methods.

56. The method of any one of claims 54 to 56, wherein the at least one signal-generating agent is covalently or non-covalently bonded to the at least one therapeutically active agent.