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(54) Title: PARTIALLY DEGRADABLE SCAFFOLDS FOR BIOMEDICAL APPLICATIONS

(57) **Abrégé/Abstract:**

The present invention is directed to a scaffold for tissue engineering, comprising a support structure having an outer surface, at least a part of the outer surface being covered by a bioactive material layer that allows cell attachment, wherein the bioactive material layer is at least partially degradable in an environment of use, to allow a detachment of the cells from the support structure by degradation of the bioactive material layer. The invention is further directed to the use of an at least partially degradable bioactive material layer on a scaffold for tissue engineering, for allowing detachment of the cells or grown tissue by degradation of the bioactive material layer.



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(54) Title: PARTIALLY DEGRADABLE SCAFFOLDS FOR BIOMEDICAL APPLICATIONS

(57) Abstract: The present invention is directed to a scaffold for tissue engineering, comprising a support structure having an outer surface, at least a part of the outer surface being covered by a bioactive material layer that allows cell attachment, wherein the bioactive material layer is at least partially degradable in an environment of use, to allow a detachment of the cells from the support structure by degradation of the bioactive material layer. The invention is further directed to the use of an at least partially degradable bioactive material layer on a scaffold for tissue engineering, for allowing detachment of the cells or grown tissue by degradation of the bioactive material layer.



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Partially degradable scaffolds for biomedical applications

Cross-reference to related applications

The present invention claims priority of U.S. provisional application serial
5 no. 60/939,607 filed May 22, 2007, the entire disclosure of which is incorporated
herein by reference.

Field of the invention

The present invention is directed to a scaffold for tissue engineering,
comprising a support structure having an outer surface, at least a part of the outer
10 surface being covered by a bioactive material layer coating that allows cell
attachment, wherein the bioactive material layer is at least partially degradable in an
environment of use, to allow a detachment of the cells from the support structure by
degradation of the bioactive material layer. The invention is further directed to the
use of an at least partially degradable bioactive material layer on a scaffold for tissue
15 engineering, for allowing detachment of the cells or grown tissue by degradation of
the bioactive material layer.

Background of the invention

Tissue engineering is an emerging discipline and field of application.
Techniques are being developed to add, modify, repair or replace cells, organized
20 cells, tissue, parts of organs or complete organs within the body of living animals or
human beings, or to provide such biological constructs outside of the living
organisms. While surgical procedures for replacement or repair of tissues or organs
may be based on artificial non-biologic substitutes, tissue engineering potentially
could provide solutions that avoid known issues of surgically used substitutes such
25 as, e. g., incompatibility of materials, foreign body reactions, wear, debris, fatigue or
fracturing. Examples for artificial surgical substitutes include heart valves, joint
implants, breast implants and the like.

Tissue engineering techniques can require the growth, induction of growth,
differentiation of, conduction of and organization of cells, organized cells, tissue, and
30 parts of organs or complete organs of the same or different organisms. Conventional
techniques can be based on providing a support structure, sometimes referred to as a
scaffold, for organization. Those scaffolds can, e.g., imitate structures for ordered
cell organization. Scaffolds can be made synthetically from biodegradable organic

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polymer materials such as, e. g., poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and their co-polymers.

Scaffolds are used to support the biologically active construct for adding, modifying, repairing or replacing cells, organized cells, tissue, parts of organs, but it
5 may also be desirable to use a scaffold that can be completely absorbed. Alternatively, cells may require external and/or mechanical, structural, electrical, chemical or other biologic signals. Deficient, insufficient or lacking signals can cause improper differentiation or dedifferentiation of cells and/or result in inappropriate or defective organization or function. For example, growth factors may be involved in
10 cell differentiation and development, for example for controlling the migration of cells, morphogenesis from one cell type to another or mitogenesis. The mode of action of growth factors can include, e. g., autocrine, on neighbouring cells, on cells far away by travelling through the bloodstream or by inducing a single cell to pass a signal onto a neighbouring cell by direct cell-cell interaction. Biologically active
15 agents such as growth factors may not only be present and used during the growth of cells, tissue or similar constructs, but also can be relevant and active for remodelling, repair or healing of injuries.

In some applications, it can be advantageous to use porous scaffolds, for example, to allow the osteoconductive growth of bone-forming cells. Also, porous
20 scaffolds can facilitate their seeding with cells, for example to allow the diffusion and/or distribution of cells into the scaffold and potentially allow a more even distribution. Replacement of bone is one application where an increasing demand may require appropriate solutions. Bone replacement can be indicated due to, e. g., trauma, infections, cancer or muscular-skeletal diseases.

25 A range of bone grafting materials have been established in clinical use, such as demineralised human bone matrix, bovine collagen mineral composites and processed coralline hydroxyapatite, calcium sulphate scaffolds, bioactive glass scaffolds and calcium phosphate scaffolds. Such orthopedic scaffolds can be used as both temporary and permanent conduits for bone. These exemplary materials can be
30 used to facilitate and direct the growth of bone or cartilage tissue across sites of

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fractures or to re-grow them in defective, damaged or infected bone. The provision of appropriate scaffolds also requires considering the structure of bone that has to be treated. Cortical and cancellous bone are structurally different, although the material composition is very similar. Cancellous bone comprises a thin interstitium lattice
5 interconnected by pores of 500-600 micron width with a spongy and open-spaced structure, whereby the interstitium can be substituted by a scaffolding material. Cortical bone comprises neurovascular "Haversian" canals of about 50-100 micron width within a hard or compact interstitium. Any suitable scaffold may allow at least osteoconduction or osteoinduction. Osteoinductive materials can actively trigger and
10 facilitate bone growth, for example by recruiting and promoting the differentiation of mesenchymal stem cells into osteoblasts. Osteoconductive materials induce bone to grow in areas where it would not normally grow, also called "ectopic" bone growth, usually by biochemical and/or physical processes. Osteogenic materials may contain cells that can form bone or can differentiate into osteoblasts.

15 Different manufacturing techniques for scaffolds and porous scaffolds have been developed and used. One conventional method of making solid scaffolds is solvent casting, *et. al.*, e. g., Mikos, *et al.*, Polymer, 1994, 35:1068-77; de Groot, *et al.*, Colloid Polym. Sci., 1991, 268:1073-81; Laurencin, *et al.*, J. Biomed. Mater. Res., 1996, 30:133-8. Others can include solvent casting and particulate leaching, melt
20 molding, fiber bonding, gas foaming or membrane lamination. For ceramic based systems also different conventional techniques such as hydrothermal conversion and burn-out of dispersed polymer phase may be used.

Conventional scaffolds have many shortcomings which may limit the use and application of tissue engineering techniques. For example, it may be desirable to
25 match the mechanical properties to the tissue that shall be replaced or regenerated, to match the degradation rate appropriately, to provide a scaffold that comprises a structure and geometry that allows growing, conducting and inducing different entities of cells, organized cells, tissue, parts of organs or organs. Other disadvantages can include that the scaffolds either can not be used for ex-vivo
30 growth or, if so, then only limited for in-vivo use. Moreover, particularly for

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scaffolds, the accurate design and distribution of pores, including the combination of different porous structures and fine-structures is significantly limited. For example, porous ceramic systems may suffer from poor control over pore size distribution, and may also exhibit limited moldability compared to polymers. Polymers, either
5 biodegradable or not, may be degraded to toxic metabolites, cause inflammation or allergic reactions or adversely interact with blood, such as triggering thrombosis.

A specific disadvantage of conventional scaffolds for ex-vivo use, e.g. in a cell cultivation system, can be that harvesting the grown tissue may be problematic because of difficulties to separate the grown tissue from the scaffold material without
10 substantially damaging cells.

Further disadvantages can include that the function of the scaffolds is only inductive or only conductive but not both. From a clinically relevant perspective, a major drawback can be that in-vivo used scaffolds are typically difficult to be detected by imaging methods during or after implantation.

15 Objects and summary of the invention

One exemplary object of the present invention is to provide a tissue engineering scaffold that can be used ex-vivo in a cell cultivation system or bioreactor for tissue engineering, and which may additionally optionally also be used in-vivo as an implantable scaffold or seed implant. Another object of the present
20 invention is to provide a class of scaffolds that can be used as complex structures for ex-vivo and/or in-vivo growth of cells, organized cells, tissue or organs or parts thereof. A further object of the present invention is to provide scaffolds for ex-vivo use, e.g. for use in a cell cultivation system, that allow harvesting or separation of the grown tissue from the scaffold material without the use of enzymes and/or essentially
25 without substantially damaging cells. A still further object of the present invention is to provide scaffolds for replacement or repair of tissues, parts of organs or organs in-vivo or ex-vivo. Another object of the present invention is to provide scaffolds wherein the mechanical, chemical, biological and physical properties such as electrical conductivity, optical or other suitable properties can be tailored
30 appropriately to the intended use. One further object of the present invention is to

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provide scaffolds that can be used for ex-vivo perfused systems, such as assisted systems, to partially or completely replace organ functions. Another object is to provide a manufacturing process for the scaffolds as described herein.

According to an exemplary embodiment of the present invention, a scaffold
5 for tissue engineering is provided, comprising a support structure having an outer surface, at least a part of the outer surface being covered by a bioactive material layer that allows cell attachment, wherein the bioactive material layer is at least partially degradable in an environment of use, to allow a detachment of the cells by degradation of the bioactive material layer.

10 According to another exemplary embodiment of the present invention, a scaffold as described above for tissue engineering is provided, which may be used in ex-vivo perfused systems, such as assisted systems, to partially or completely replace organ functions.

According to another exemplary embodiment of the present invention the use
15 of an at least partially degradable bioactive material layer on the surface of a support structure of a scaffold for tissue engineering for separating tissue grown on the scaffold by degrading the bioactive material layer in an environment of use is provided.

According to a further exemplary embodiment of the present invention a
20 method for tissue engineering is provided, comprising the steps of providing, in a cell culture system or bioreactor, a scaffold comprising a support structure having an outer surface, at least a part of the outer surface being covered with a bioactive material layer that allows cell attachment, wherein the bioactive material is at least partially degradable in an environment of use to allow a detachment of the cells from
25 the support structure by degradation of the bioactive material layer; inoculating the scaffold with cells or living tissue; cultivating the inoculated scaffold in a suitable environment to grow tissue; and harvesting the grown tissue after and/or by optionally actively induced degradation of the bioactive layer.

For example, the environment of use may be an ex-vivo environment which
30 may include e.g. a liquid medium, such as a cell culture medium or a nutritional

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medium, a solvent or solvent system, or even an at least partially gaseous medium. Alternatively or additionally, the environment of use may include an in-vivo environment inside the human or animal body which is exposed to body fluids such as blood, blood plasma, lymph, bile, urine. In particular, such environments may
5 include extra-cellular fluids such as organ-specific plasma, interstitial and transcellular fluids. Transcellular fluids for example include the digestive secretions (within glands and ducti), cerebrospinal, intraocular, pleural, pericardial, peritoneal, seminal and synovial fluids, cochlear endolymph and the secretions of glands.

The at least partially degradable bioactive material layer is typically provided
10 in the form of a coating which covers the outer surface of the scaffold's supporting structure at least partially, or completely. The at least partially degradable bioactive material layer allows a simple and efficient separation of the tissue grown on the scaffold, since the bioactive layer can be degraded over time in the environment of use, so that the tissue grown may be harvested easily in one homogeneous part or
15 layer without damages after a certain time essentially determined by the composition of the layer and/or the environment of use. For example, the tissue may be incrementally or gradually loosened from the underlying support structure or an interconnecting cell layer. Alternatively, the bioactive material layer can be made of a material that at least partially degrades after degradation has been actively induced,
20 for example by applying an electrical current, a pH-change in the medium or the like. Thus, in accordance with exemplary embodiments of the present invention, problems conventionally associated with separating a grown tissue from e.g. complex shaped supports without substantially damaging the tissue can be successfully overcome. Also, the use of expensive enzymes to separate tissue from the support can be
25 avoided.

The scaffolds may be used for growing biologic constructs made of all types of cells and organs, including, for example, but not limited to, hepatocytes, pancreatic islet cells, fibroblasts, chondrocytes, osteoblasts, exocrine cells, cells of intestinal origin, bile duct cells, parathyroid cells, thyroid cells, cells of the adrenal-
30 hypothalamic-pituitary axis, heart muscle cells, kidney epithelial cells, kidney

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tubular cells, kidney basement membrane cells, nerve and neural cells, blood vessel cells, cells forming bone and cartilage, smooth muscle cells, skeletal muscle cells, ocular cells, integumentary cells, keratinocytes or stem cells of mammal, in particular human origin. The biologic constructs can include cells and compounds
5 from any desired species or any combination thereof, including genetically modified biologic material.

A scaffold providing in accordance with exemplary embodiments can be tailored to have conductive or inductive or combined properties for growing cells and tissues. Such scaffold may also comprise rationally designed structures to allow
10 engraftment, ingrowth, induction or conduction of attached cells or tissues or any combination thereof. For example, as desired for a particular application, the support may have a complex structure, e.g. osteoconductive, structure while it is still possible to easily detach the grown tissue from the support by inducing degradation of the bioactive material layer covering the support and forming the physical link between
15 the support and the adherent cells or tissue grown there upon.

A further aspect is that the present invention comprises a class of scaffolds that can incorporate and/or release or absorb beneficial agents useful for growth, induction or conduction, differentiation or dedifferentiation of cells and living tissue.

Definitions

20 The terms "biodegradable" or "degradable" as used herein refer to any material which can be removed in-vivo or ex-vivo in an environment of use, e.g. by (bio)corrosion or (bio)degradation. Thus, any material, e.g. a metal or organic polymer that can be degraded, absorbed, metabolized, or which is resorbable in the human or animal body, in a cell culture system or bioreactor may be used in the
25 embodiments of the present invention. Also, as used in this description, the terms "biodegradable", "bioabsorbable", "resorbable", and "biocorrodible" refer to materials that are broken down and may be gradually absorbed or eliminated, regardless whether these processes are due to hydrolysis, metabolic processes, bulk or surface erosion.

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The term "biological construct" as used herein refers to and can be used as a synonym for cells, any agglomeration of cells or tissue which may be grown on the scaffold and harvested from it, specifically living cells, organized cells, living tissue, and parts of organs or complete organs of the same entity or different entities,
5 particularly of mammals, animals and human origin, also including cells and tissue having been genetically modified.

The terms "active ingredient", "active agent" or "beneficial agent" as used herein can include any material or substance which may be used to add a function to the scaffold. Examples of such active ingredients include biologically,
10 therapeutically or pharmacologically active agents such as drugs or medicaments, diagnostic agents such as markers, or absorptive agents. The active ingredients may be a part of the support structure or the bioactive material layer, such as incorporated into the scaffold or being coated on at least a part of the scaffold. Biologically or therapeutically active agents comprise substances being capable of providing a direct
15 or indirect therapeutic, physiologic and/or pharmacologic effect in a human or animal organism. A therapeutically active agent may include a drug, pro-drug or even a targeting group or a drug comprising a targeting group. An "active ingredient" may further include a material or substance which may be activated physically, e.g. by radiation, or chemically, e.g. by metabolic processes.

20 The term "porous" as used herein designates a property of a material, which is determined by the presence of a plurality of interconnected pores. The volume of the pores can be assessed by measuring the porosity of the material as conventionally known, e. g. by N₂-adsorption methods such as BET and further defined herein. "Porous" does not include holes such as boreholes or the like.

25 The term "support structure" is used to designate the bulk structure of the scaffold, i.e. the device body. To the contrary, a coating cannot be a part of a support structure.

Exemplary embodiments of the present invention will now be described in greater detail. The following description makes reference to numerous specific
30 details in order to provide a thorough understanding of the present invention.

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However, each and every specific detail needs not to be employed to practice the present invention. Also, the features of all exemplary embodiments are principally combinable with each other, if not expressly stated otherwise.

Detailed description of preferred embodiments

5 In an exemplary embodiment of the invention a scaffold is provided which includes a support structure which is at least partially covered on its outer and/or inner surfaces with a bioactive and at least partially degradable material layer. The degradable bioactive material layer can allow a separation of the tissue grown on the scaffold while it is degraded over time in the environment of use, or its degradation
10 can be induced by a stimulus, so that the tissue grown may be harvested easily in one homogeneous part or layer without significant damages, by reducing or eliminating the physical contact between the tissue and the scaffold's support structure or the bioactive material layer on its outer surface. Such exemplary embodiments of the present invention can overcome the problems conventionally associated with
15 separating the grown tissue from e.g. complex shaped supports essentially without damaging the tissue. Also, the use of expensive enzymes to separate tissue from the support can be avoided.

The support structure may have any desired shape or form, depending on the specific application, suitable for growing cells or tissue on it. For example, the
20 support structure may have a honeycomb, mesh or tubular structure for ex-vivo cell culturing systems, or may be in the form of a "must-fit" implant for replacement of bone or cartilage, which may be implanted into the human or animal body after tissue has been grown on the scaffold in an ex-vivo culturing system. Basically, the scaffold can be made from one part or from an assembly of multiple parts as desired.

25 *Support structure*

The support structure may consist of or include materials such as, e.g. inorganic, organic or mixed inorganic/organic hybrid materials, including materials as conventionally used for cell culture supports or tissue engineering scaffolds, which may be porous or non-porous, and may be structured or designed as desired
30 for the intended application. The support structure can be made from the same or a

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different material than the bioactive material layer, typically from a different material, so that the materials discussed below in the context of the support structure may also be used in the bioactive material layer and vice versa, with the prerequisite that the material of the bioactive material layer is at least partially degradable in an environment of use as described herein. Also, the support structure material and the bioactive material layer may be both degradable, preferably having different degradation rates/properties. In exemplary embodiments, the support structure is made of a substantially non-degradable material.

For example, the support structure can be made from at least one of an inorganic material, an organic material, an inorganic-organic hybrid material, a carbon material, a polymer material, a ceramic material, a metal or metal alloy material, or any composites or combinations thereof.

In an exemplary embodiment of the present invention the support structure consists of or includes organic materials. Such materials can include biocompatible polymers, oligomers, or pre-polymerized forms as well as polymer composites which may include at least partially degradable materials. The polymers used may be thermosets, thermoplastics, synthetic rubbers, extricable polymers, injection molding polymers, moldable polymers, spinnable, weavable and knittable polymers, oligomers or pre-polymerizes forms and the like or mixtures thereof. In exemplary embodiments, the material of the support structure can include organic materials being biodegradable per se, such as, but not limited to, collagen, albumin, gelatin, hyaluronic acid, starch, cellulose (methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulose-phtalate); furthermore casein, dextrane, polysaccharide, fibrinogen, poly(D,L lactide), poly(D,L-lactide-Co-glycolide), poly(glycolide), poly/hydroxybutylate), poly(alkylcarbonate), poly(orthoester), polyester, poly(hydroxyvaleric acid), polydioxanone, poly(ethylene terephthalate), poly(maleic acid), poly(tartaric acid), polyanhydride, polyphosphazene, poly(amino acids), and/or copolymers or mixtures thereof.

In another exemplary embodiment the support structure is based on inorganic composites, organic composites or hybrid inorganic/organic composites.

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In one exemplary embodiment, the support structure of the scaffold includes or consists of glassy carbon or vitreous carbon, e.g., a non-graphitizing type of inorganic carbon material, which combines glassy and ceramic properties with those of graphite. Important properties of this material are, e.g. its biocompatibility, bio-
5 inertness, and resistance to chemical attack. Glassy carbon is a conventional material, widely used, e.g., as an electrode material in electrochemistry, and may be produced from organic precursor materials such as polymers or phenolic resins at temperatures up to 3000 °C by carbonization, and may further be widely varied in its physical properties.

10 The structure of glassy carbon can be 100% sp^2 -hybridized carbon, e.g., a graphite or fullerene like structure. Certain molecular models assumed that both sp^2 and sp^3 -bonded atoms may be present. A later model was based on the assumption that the molecular orientation of the polymeric precursor material can be “memorized” to some extent after carbonization. Thus, the structure may bear some
15 resemblance to that of a polymer, in which the "fibrils" can be very narrow curved and twisted ribbons of graphitic, and thus inorganic, carbon. However, more recent research has suggested that glassy carbon has a fullerene-related structure. For example, glassy or vitreous carbon can include two-dimensional structural elements (sp^2 -C) and does not exhibit ‘dangling’ bonds, such as e.g. amorphous carbon does.

20 The support structure may include amorphous carbon, e.g., a glassy carbon material that essentially does not have any crystalline structure, but can include a certain amount of sp^3 -carbon structural elements. Amorphous carbon can reveal some short-range order, but there may be no long-range pattern of atomic positions.

In further exemplary embodiments, the support structure may include
25 diamond-like carbon (DLC) which is also an amorphous carbon material that can display some of the properties of diamond. Such materials may contain significant amounts, for example, up to 100 %, of sp^3 hybridized carbon atoms, wherein the carbon atoms may be arranged in a cubic lattice or a hexagonal lattice, or mixtures thereof. Furthermore, mixtures of amorphous, diamond-like, vitreous, glassy, or

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other carbon materials may be used for preparing the support structure of exemplary scaffolds of the present invention.

Optionally, the carbon material, such as amorphous, diamond-like, vitreous or glassy carbon material may be mixed with other materials such as metals, alloys, ceramic, polymers, or the like, preferably in minor amounts, e.g. less than 30 % by weight, preferably less than 10 % by weight. According to exemplary embodiments of the present invention the optionally porous scaffolds may have a carbon content of at least about 20% by weight, preferably sp^2 carbon or, in specific embodiments, sp^3 carbon or any mixture thereof. Inorganic materials with sp^2 carbon or sp^3 carbon contacted with physiologic fluids or living cells or tissue exhibit bioinert or bioactive properties and can be superior to other materials in terms of cytotoxicity, haemocompatibility, inflammation or engraftment and respective tissue or cell adhesion.

Different materials may also be used, e. g., to provide or form different sections or parts of the scaffold support structures. According to an exemplary embodiment of the invention, the support structure includes a composite material comprising inorganic carbon as described above, and a further inorganic material selected from, e.g., at least one of a metal, a metal alloy, or a metal compound.

According to an exemplary embodiment, an optionally porous support structure can consist of or include a metal compound, a metal or metal alloys, e.g. metals and metal alloys selected from main group metals of the periodic system, transition metals such as copper, gold and silver, titanium, zirconium, hafnium, vanadium, niobium, tantalum, chromium, molybdenum, tungsten, manganese, rhenium, iron, cobalt, nickel, ruthenium, rhodium, palladium, osmium, iridium or platinum, or from rare earth metals. For example, the metal compound may include zero-valent metals, metal oxides, metal carbides, metal nitrides, metal oxynitrides, metal carbonitrides, metal oxycarbides, metal oxynitrides, metal oxycarbonitrides and the like, and any mixtures thereof. The metals or metal oxides or alloys used may also be magnetic. Examples include iron, cobalt, nickel, manganese and mixtures thereof, for example iron, platinum mixtures or alloys, or for example, magnetic

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metal oxides such as iron oxide and ferrite. Semi-conducting materials or alloys may also be used, for example semi-conductors from Groups II to VI, Groups III to V, and Group IV. Suitable Group II to VI semi-conductors include, for example, MgS, MgSe, MgTe, CaS, CaSe, CaTe, SrS, SrSe, SrTe, BaS, BaSe, BaTe, ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe, or mixtures thereof. Examples for suitable Group III to V semi-conductors are GaAs, GaN, GaP, GaSb, InGaAs, InP, InN, InSb, InAs, AlAs, AlP, AlSb, AIS and mixtures thereof. Examples for Group IV semi-conductors include germanium, lead and silicon. The semi-conductors may also comprise mixtures of semi-conductors from more than one group and all the groups mentioned above are included.

Metal compounds which may be used can include metals or metal-oxides or alloys that comprise MRI visibility or radiopacity, preferably implants made from ferrite, tantalum, tungsten, gold, silver or any other suitable metal, metal oxide or alloy, such as platinum-based radiopaque steel alloys, so-called PERSS (platinum-enhanced radiopaque stainless steel alloys), cobalt alloys or any mixture thereof. In such embodiments, the scaffold can be detectable by non-invasive diagnostic methods, e.g., if used in-vivo.

Exemplary, support structure may also include a combination or composite of carbon materials such as those described herein, together with a metal or metal alloy as described above.

In certain embodiments, the material of the support structure can be carbon-based, i.e. having a carbon content of at least 50% or more. For example, such carbon-based material can be made by using discrete carbon particles. Such particles can include tubes, fibers, fibrous materials or wires or spherical or dendritic or any regular or irregular particle form and the preferred particle sizes are in, but not limited to, a range of 1nm up to 1000µm. Suitable particles can include carbon species such as fullerenes, in particular C36, C60, C70, C76, C80, C86, C112 etc., or any mixtures thereof, , nanotubes such as MWNT, SWNT, DWNT, random-oriented nanotubes, so-called fullerene onions or metallo-fullerenes, also graphite fibers, or particles or diamond particles. The material can be a composite, for example a

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carbon material combined with a polymer, metal or metal alloy, ceramic or bio-ceramic, mineral or any mixture thereof.

In an exemplary embodiment, the scaffold can be formed from a biodegradable metal, alloy or metal composite as described in further detail below
5 with respect to the bioactive material layer, for example, an alloy based on magnesium or calcium. Such scaffold can be primarily degraded to hydroxyl apatite within a medium or living body. This property can be especially advantageous for scaffolds with a temporary function. By alloying the aforesaid metals it is possible to control the physiologic or ex-vivo degradation rate from a few days up to 20 years.
10 Moreover, by introducing precious metals, either within the alloy, or as a part of the scaffold, either ex-vivo or in-vivo, or alternatively by applying a current or voltage, for example, with an appropriate electrode or similar device, the degradation can be substantially altered. Using a metal also can allow to utilize the mechanical strength of these compounds and to provide a tailored scaffold that can both satisfy any
15 mechanical requirements as well as being biodegradable.

In an exemplary embodiment, the composition of the materials for the support structure as described above is selected such that the degradation rate of the support structure is lower, e.g., by about 10 %, 20 %, 50% or even 100 % lower than the degradation rate of the bioactive material layer, to allow a detachment of the grown
20 tissue before the support is completely degraded.

In other embodiments the scaffold can be formed using a composite comprising at least about 10% of a degradable metal composition together with a polymer or polymer mixture that can be degradable or not, or with a ceramic, also degradable or not, or any mixture thereof.

25 The scaffold can also be made using an inorganic or an organic material, such as, e. g., a metal, a ceramic material, or a composite such as an inorganic-organic hybrid material, as further defined herein below, either degradable or not.

The scaffold material can also include materials that are capable of absorbing specific compounds or of exchanging ions, or diagnostic markers and the like, as
30 further described herein below.

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Bioactive material layer

A scaffold of exemplary embodiments of the present invention includes at least one bioactive surface that allows cell attachment. The bioactive material layer can cover the surface of the support partially or completely. To facilitate detachment
5 of grown tissue, at least a portion of the bioactive surface of the scaffold includes a bioactive material layer that is at least partially degradable in an environment of use, e.g. a cell culture medium or a body fluid, as described above. The bioactive material layer can comprise a patch-like composite of degradable and non-degradable structures and materials. Degradation is useful, e.g. if the biologic construct has to be
10 detached without using enzymes that affect the vitality or the structure of such biologic constructs or tissue. For example, controlled detachment of biologic constructs by applying enzymes in conventional complex formed or shaped scaffolds with porous structures can be difficult.

In further exemplary embodiments, the bioactive material layer consists of or
15 includes, attached to the support structure, an at least partially degradable or corrodible layer of an inorganic material such as a solid metal or metal alloy, or optionally metal or metal alloy particles embedded in a matrix material, e.g. an alkoxide-based gel or a polymer. For example, the at least partially degradable layer may consist of or include biodegradable metals or metal oxides, carbides, nitrides
20 and mixed forms thereof, or metal alloys, including alkaline or alkaline earth metals, Fe, Zn or Al, such as Mg, Fe or Zn, and optionally alloyed with or combined with other metals selected from Mn, Co, Ni, Cr, Cu, Cd, Pb, Sn, Th, Zr, Ag, Au, Pd, Pt, Si, Ca, Li, Al, Zn and/or Fe. Other suitable materials include, e.g., alkaline earth metal oxides or hydroxides such as magnesium oxide, magnesium hydroxide,
25 calcium oxide, and calcium hydroxide or mixtures thereof.

In exemplary embodiments, the degradable bioactive material layer can include biodegradable or biocorrosive metals or alloys based on at least one of magnesium or zinc, or an alloy comprising at least one of Mg, Ca, Fe, Zn, Al, W, Ln, Si, or Y. Furthermore, the bioactive material layer may be substantially completely
30 or at least partially degradable in-vivo or ex-vivo as described herein. Examples for

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suitable biodegradable alloys include e.g. magnesium alloys comprising more than 90 % of Mg, about 4-5 % of Y, and about 1.5-4 % of other rare earth metals such as neodymium and optionally minor amounts of Zr; or biocorrosive alloys comprising as a major component tungsten, rhenium, osmium or molybdenum, for example
5 alloyed with cerium, an actinide, iron, tantalum, platinum, gold, gadolinium, yttrium or scandium.

The biodegradable metal or metal alloy may include in an exemplary embodiment

(i) About 10-98 wt.-%, about 35-75 wt.-% of Mg, and about 0-70 wt.-%, or
10 about 30-40% of Li and about 0-12wt.-% of other metals, or

(ii) About 60-99wt.-% of Fe, about 0.05-6wt.-% Cr, about 0.05-7wt.-% Ni and up to about 10wt.-% of other metals; or

(iii) About 60-96wt.-% Fe, about 1-10wt.-% Cr, about 0.05-3wt.-% Ni and about 0-15wt.-% of other metals, wherein component percentages selected for a
15 particular alloy add up to 100 %.

In further exemplary embodiments, the bioactive material layer may include particles of the above mentioned biodegradable metallic materials embedded in e.g. a polymeric matrix or a sol/gel derived matrix which may itself be degradable or not. Degradation of the metallic particles in the bioactive layer allows a partial
20 degradation of the layer where the particles are exposed to the medium and/or biological cells, resulting in the loss of "anchoring sites" for the cells or tissue, thus leading to a detachment of the tissue from the support.

In other preferred embodiments, detachment is achieved by bulk degradation of the bioactive material layer or parts of the bioactive layer. Specifically, the
25 bioactive layer can be partially or completely made from magnesium, magnesium compounds or magnesium alloys. In one example, the bioactive layer is a composite material of an inorganic or organic polymer or sol/gel derived matrix comprising magnesium or magnesium alloy particles, preferably in a particle size range of 100nm to 2000 μ m, more referred from 200nm to 10 μ m and most preferred from
30 200nm to 1 μ m. In physiologic fluids or suitably selected liquid media, the

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magnesium or magnesium alloys will be degraded to degradation products such as hydroxyl apatite and hydrogen gas. If partially or completely used in or for the bioactive material layer, the degradation of the magnesium-based materials or other degradable metal materials over time will result in a loss of integrity of the bioactive layer and allow mechanical removal of previously attached cells or biological constructs. In specifically preferred embodiments the magnesium, magnesium compounds or magnesium alloys are used as particulates within a bioactive layer, preferably with a volume content of 10 to 90 %, more preferred from 20% to 90% and most preferred from 30% to 60% of degradable metallic or metal-based particles, e.g. magnesium particles, in a polymeric or sol/gel-derived matrix. Adhesion of cells and biological constructs will then be affected over time due to a loss of connective surface. Preferred materials for use as the matrix for such composite bioactive layers are biocompatible polymers such as poly(lactic) acid (PLA) or PGLA, and the other biocompatible and/or biodegradable polymers mentioned herein below. In further embodiments the bioactive material layer is completely made out of magnesium, magnesium compounds or magnesium alloys, for example using thin metallic foils at least partially covering the support.

The bioactive material layer may also consist of or comprise, for example as a matrix of a composite material, an organic material such as a polymer that can be degraded ex-vivo or in-vivo, and such layer can be used to detach the biologic construct ex-vivo. Useful polymers can include pH-sensitive polymers, shape memory polymers and the like. The polymers used should be biocompatible. In exemplary embodiments, it can be particularly preferred to select polymers from pH-sensitive polymers, such as: homopolymers such as poly(amino carboxylic acid), poly(acrylic acid), poly(methyl acrylic acid), and their copolymers. This applies likewise for polysaccharides such as celluloseacetatephthalate, hydroxylpropylmethylcellulose-phthalate, hydroxypropylmethylcellulosesuccinate, celluloseacetate trimellitate and chitosan. Further polymers may be selected from temperature sensitive polymers, such as, for example, however not exclusively: poly(N-isopropylacrylamide-co-sodium-acrylate-co-n-N-alkylacrylamide), poly(N-

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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 suitable to be used as polymers with thermogel characteristics are hydroxypropylcellulose, methylcellulose, hydroxypropylmethylcellulose, ethylhydroxyethylcellulose and pluronics such as F-127, L-122, L-92, L-81, L-61. Preferred polymers include also, however not exclusively, functionalized styrene, such as amino styrene, functionalized dextrane and polyamino acids. Furthermore, 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 may be used. 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.

In exemplary embodiments, the material for the bioactive material layer can include organic materials being biodegradable per se, for example collagen, albumin, gelatin, hyaluronic acid, starch, cellulose (methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulose-phtalate); furthermore casein, dextrane, polysaccharide, fibrinogen, poly(D,L lactide), poly(D,L-lactide-Coglycolide), poly(glycolide), poly(hydroxybutylate), poly(alkylcarbonate), poly(orthoester), polyester, poly(hydroxyvaleric acid), polydioxanone, poly(ethylene, terephthalate), poly(maleic acid), poly(tartaric acid), polyanhydride, polyphosphazene, poly(amino acids), and all of the copolymers and any mixtures thereof. Such polymeric materials may also be used in the bioactive material layer as a matrix material, e.g., to embed biodegradable metallic or other particles as described above, thus providing a composite bioactive material layer.

In further exemplary embodiments, the bioactive material layer may include particles of the above mentioned biodegradable polymer materials embedded in e.g. a metallic matrix or a sol/gel derived matrix or combined with metallic particles, which

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may all itself be degradable or not. Degradation of the polymeric particles in the bioactive layer allows a partial degradation thereof, resulting in the loss of "anchoring sites" for the cells or tissue, thus leading to a detachment of the tissue from the support.

5 In further exemplary embodiments, the bioactive material layer may comprise or consist of an inorganic-organic hybrid material or a ceramic material. For example, the hybrid material can be obtained using a sol-gel processing technique and may include a gel obtained by hydrolysis of a reaction mixture comprising a silicon alkoxide compound. For example, the reaction mixture may further include a
10 carbon material selected from at least one of fullerenes, for example, C36, C60, C70, C76, C80, C86, C112 etc., or any mixtures thereof, carbon nanotubes such as MWNT, SWNT, DWNT, random-oriented nanotubes, as well as so-called fullerene onions or metallo-fullerenes, soot, lamp black and the like. Carbon fibers, diamond particles or graphite particles may also be used.

15 Biocompatibility of such carbon-based materials may be used to promote cell adhesion to the bioactive material layer, and the typically non-degradable carbon species can be embedded in a material that is at least partially degradable.

 Sol/gel technology allows for the production of highly biocompatible, in some instances bioerodible or biodegradable materials at low temperatures. Sol/gel-
20 process derived materials may be used to form at least partially degradable bioactive material layers, or these materials may be used to form suitable matrices for degradable particles as described above, suitable for providing the bioactive material layer. Additionally, sol/gel-derived materials may be easily applied to a support in the form of a liquid sol coating, which is then cured to form the bioactive layer.

25 The sol/gel-process technology can be widely applied to build up different types of networks. The linkage of the components under formation of the sol or gel can take place in several ways, e.g. via conventional hydrolytic or non-hydrolytic sol/gel-processing, and may be used to produce, e.g., aerogels or xerogels.

 A "sol" is a dispersion of colloidal particles in a liquid, and the term "gel"
30 connotes an interconnected, rigid network of pores of typically sub micrometer

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dimensions and polymeric chains whose average length is typically greater than a micrometer. For example, the sol/gel-process may involve mixing of the precursors, e.g. a sol/gel forming components into a sol, adding further additives or materials, casting the mixture in a mould or applying the sol onto a substrate in the form of a coating, gelation of the mixture, whereby the colloidal particles are linked together to become a porous three-dimensional network, aging of the gel to increase its strength; converting the gel into a solid material by drying from liquid and/or dehydration or chemical stabilisation of the pore network, and densification of the material to produce structures with ranges of physical properties. Such processes are described, for example, in Henge and West, The Sol/Gel-Process, 90 Chem. Ref. 33 (1990).

The term "sol/gel" as used within this specification may mean either a sol or a gel. The sol can be converted into a gel as mentioned above, e.g. by aging, curing, raising of pH, evaporation of solvent or any other conventional methods, which can result in inorganic-organic hybrid materials or ceramic materials.

The term semi-solid refers to materials having a gel-like consistency, i.e. being substantially dimensionally stable at room temperature, but have a certain elasticity and flexibility, typically due to a residual solvent content.

The bioactive material layer may be produced from a sol which is subsequently being converted into a solid or semi-solid material layer. The sol can be prepared from any type of sol/gel forming components in a conventional manner. The skilled person will –depending on the desired properties and requirements of the material to be produced – select the suitable components / sols for preparing the bioactive material layer based on his professional knowledge.

The sol/gel forming components can include, e. g., alkoxides, oxides, acetates, nitrates of various metals, e.g. silicon, aluminum, boron, magnesium, zirconium, titanium, alkaline metals, alkaline earth metals, or transition metals, and from platinum, molybdenum, iridium, tantalum, bismuth, tungsten, vanadium, cobalt, hafnium, niobium, chromium, manganese, rhenium, iron, gold, silver, copper, ruthenium, rhodium, palladium, osmium, lanthanum and lanthanides, as well as combinations thereof.

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In some exemplary embodiments of the present invention, the sol/gel forming components can be selected from metal oxides, metal carbides, metal nitrides, metaloxynitrides, metacarbonitrides, metaloxycarbides, metaloxynitrides, and metaloxycarbonitrides of the above mentioned metals, or any combinations thereof.

5 These compounds, which may be in the form of colloidal particles, can be reacted with oxygen containing compounds, e.g. alkoxides to form a sol/gel, or may be added as fillers if not in colloidal form.

In other exemplary embodiments of the present invention, the sols may be derived from at least one sol/gel forming component selected from alkoxides, metal
10 alkoxides, colloidal particles, particularly metal oxides and the like. The metal alkoxides that may be used as sol/gel forming components may be conventional chemical compounds that may be used in a variety of applications. These compounds have the general formula $M(OR)_x$ wherein M is any metal from a metal alkoxide which e.g. may hydrolyze and polymerize in the presence of water. R is an alkyl
15 radical of 1 to 30 carbon atoms, which may be straight chained or branched, and x has a value equivalent to the metal ion valence. Metal alkoxides such as $Si(OR)_4$, $Ti(OR)_4$, $Al(OR)_3$, $Zr(OR)_3$ and $Sn(OR)_4$ may be used. Specifically, R can be the methyl, ethyl, propyl or butyl radical. Further examples of suitable metal alkoxides can include $Ti(isopropoxy)_4$, $Al(isopropoxy)_3$, $Al(sec-butoxy)_3$, $Zr(n-butoxy)_4$ and
20 $Zr(n-propoxy)_4$.

In exemplary embodiments, the sols are based on silanes or alkoxide compounds, e.g., being made from silicon alkoxides such as tetraalkoxysilanes, wherein the alkoxy may be branched or straight chained and may contain 1 to 25 carbon atoms, e.g. tetramethoxysilane (TMOS), tetraethoxysilane (TEOS) or tetra-n-propoxysilane, as well as oligomeric forms thereof. Also suitable are alkylalkoxy-
25 silanes, wherein alkoxy is defined as above and alkyl may be a substituted or unsubstituted, branched or straight chain alkyl having about 1 to 25 carbon atoms, e.g., methyltrimethoxysilane (MTMOS), methyltriethoxysilane, ethyltriethoxysilane, ethyltrimethoxysilane, methyltripropoxysilane, methyltributoxysilane, propyltri-
30 methoxysilane, propyltriethoxysilane, isobutyltriethoxysilane, isobutyltri-

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methoxysilane, octyltriethoxysilane, octyltrimethoxysilane, which is commercially available from Degussa AG, Germany, methacryloxydecyltrimethoxysilane (MDTMS); aryltrialkoxysilanes such as phenyltrimethoxysilane (PTMOS), phenyltriethoxysilane, which is commercially available from Degussa AG, Germany;
5 phenyltripropoxysilane, and phenyltributoxysilane, phenyl-tri-(3-glycidyloxy)-silane-oxide (TGPSO), 3-aminopropyltrimethoxysilane, 3-aminopropyl-triethoxysilane, 2-aminoethyl-3-aminopropyltrimethoxysilane, triaminofunctional propyltrimethoxysilane (Dynasylan[®] TRIAMO, available from Degussa AG, Germany), N-(n-butyl)-3-aminopropyltrimethoxysilane, 3-aminopropylmethyl-diethoxysilane, 3-
10 glycidyloxypropyltrimethoxysilane, 3-glycidyloxypropyltriethoxy-silane, vinyltrimethoxysilane, vinyltriethoxysilane, 3-mercaptopropyltrimethoxy-silane, Bisphenol-A-glycidylsilanes; (meth)acrylsilanes, phenylsilanes, oligomeric or polymeric silanes, epoxysilanes; fluoroalkylsilanes such as fluoroalkyltrimethoxysilanes, fluoroalkyltriethoxysilanes with a partially or fully fluorinated, straight chain
15 or branched fluoroalkyl residue of about 1 to 20 carbon atoms, e.g. tridecafluoro-1,1,2,2-tetrahydrooctyltriethoxysilane and modified reactive fluoroalkylsiloxanes which are available from Degussa AG under the trademarks Dynasylan[®] F8800 and F8815; as well as any mixtures of the foregoing. Such sols may be easily converted into solid porous aerogels by drying.

20 In another exemplary embodiment of the present invention, the sol may be prepared from carbon-based nano-particles and organic alkaline or earth alkaline metal salts, e.g. their formiates, acetates, propionates, malates, maleates, oxalates, tartrates, citrates, benzoates, salicylates, phtalates, stearates, phenolates, sulfonates, and amines, as well as acids, such as phosphorous acids, pentoxides, phosphates, or
25 organo phosphorous compounds such as alkyl phosphonic acids. Further substances that may be used to form sols for e.g. bioerodible or degradable bioactive material layers include sols made from magnesium acetate, calcium acetate, phosphorous acid, P₂O₅ as well as triethyl phosphite as a sol in ethanol or ethanediol, whereby biodegradable composites can be prepared from physiologically acceptable organic
30 or inorganic components. For example, by varying the stoichiometric Ca/P-ratio, the

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degeneration rate of such composites can be adjusted. A molar ratio of Ca to P can be about 0.1 to 10, or preferably about 1 to 3.

In some exemplary embodiments of the present invention, the sols can be prepared from colloidal solutions, which may comprise carbon-based nanoparticles, preferably in solution, dispersion or suspension in polar or nonpolar solvents, including aqueous solvents as well as cationically or anionically polymerizable polymers as precursors, such as alginate. By addition of suitable coagulators, e.g. inorganic or organic acids or bases, including acetates and diacetates, carbon containing composite materials can be produced by precipitation or gel formation. Optionally, further additives can be added to adjust the properties of the resultant drug delivery material.

The sol/gel components used in the sols may also comprise colloidal metal oxides, preferably those colloidal metal oxides which are stable long enough to be able to combine them with the other sol/gel components and the polymer-encapsulated active agents. Such colloidal metal oxides may include, but are not limited to, SiO_2 , Al_2O_3 , MgO , ZrO_2 , TiO_2 , SnO_2 , ZrSiO_4 , B_2O_3 , La_2O_3 , Sb_2O_5 and $\text{ZrO}(\text{NO}_3)_2$. SiO_2 , Al_2O_3 , ZrSiO_4 and ZrO_2 may be preferably selected. Further examples of the at least one sol/gel forming component include aluminumhydroxide sols or -gels, aluminumtri-sec-butylat, AlOOH -gels and the like.

Such colloidal sols may be acidic in the sol form and, therefore, when used during hydrolysis, it may not be necessary to add additional acid to the hydrolysis medium. These colloidal sols can also be prepared by a variety of methods. For example, titania sols having a particle size in the range of about 5 to 150 nm can be prepared by the acidic hydrolysis of titanium tetrachloride, by peptizing hydrous TiO_2 with tartaric acid and, by peptizing ammonia washed $\text{Ti}(\text{SO}_4)_2$ with hydrochloric acid. Such processes are described, for example, by Weiser in Inorganic Colloidal Chemistry, Vol. 2, p. 281 (1935). To preclude the incorporation of contaminants in the sols, the alkyl orthoesters of the metals can be hydrolyzed in an acid pH range of about 1 to 3, in the presence of a water miscible solvent, wherein

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the colloid is present in the dispersion in an amount of about 0.1 to 10 weight percent.

Where the sol is formed by a hydrolytic sol/gel-process, the molar ratio of the added water and the sol/gel forming components, such as alkoxides, oxides, acetates, nitrides or combinations thereof may be in the range of about 0.001 to 100, preferably from about 0.1 to 80, more preferred from about 0.2 to 30.

In a typical hydrolytic sol/gel processing procedure which can be used in exemplary embodiments of the invention, the sol/gel components are optionally blended with (optionally chemically modified) fillers such as biodegradable particles in the presence of water, applied to the surface of the support structure and cured. Optionally, further solvents or mixtures thereof, and/or further additives may be added, such as surfactants, fillers and the like. The solvent may contain salts, buffers such as PBS buffer or the like to adjust the pH value, the ionic strength etc. Further additives such as cross linkers may be added, as well as catalysts for controlling the hydrolysis rate of the sol or for controlling the cross linking rate. Non-hydrolytic sols may be similarly made, but likely essentially in the absence of water.

When the sol is formed by a non-hydrolytic sol/gel-process or by chemically linking the components with a linker, the molar ratio of the halide and the oxygen-containing compound may be in the range of about 0.001 to 100, or preferably from about 0.1 to 140, even more preferably from about 0.1 to 100, particularly preferably from about 0.2 to 80.

In non-hydrolytic sol/gel processes, the use of metal alkoxides and carboxylic acids and their derivatives may also be suitable. Suitable carboxylic acids include acetic acid, acetoacetic acid, formic acid, maleic acid, crotonic acid, succinic acid, their anhydrides, esters and the like.

Non-hydrolytic sol/gel processing in the absence of water may be accomplished by reacting alkylsilanes or metal alkoxides with anhydrous organic acids, acid anhydrides or acid esters, or the like. Acids and their derivatives may be suitable as sol/gel components and/or for modifying/functionalizing the encapsulated active agents.

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In certain exemplary embodiments of the present invention, the sol may also be formed from at least one sol/gel forming component in a non-hydrous sol/gel processing, and the reactants can be selected from anhydrous organic acids, acid anhydrides or acid esters such as formic acid, acetic acid, acetoacetic acid, succinic acid, maleic acid, crotonic acid, acrylic acid, methacrylic acid, partially or fully fluorinated carboxylic acids, their anhydrides and esters, e.g. methyl- or ethylesters, and any mixtures of the foregoing. It is often preferred to use acid anhydrides in admixture with anhydrous alcohols, wherein the molar ratio of these components determines the amount of residual acetoxo groups at the silicon atom of the alkylsilane employed.

Typically, according to the degree of cross linking desired in the resulting sol, either acidic or basic catalysts may be applied, particularly in hydrolytic sol/gel processes. Suitable inorganic acids include, for example, hydrochloric acid, sulfuric acid, phosphoric acid, nitric acid as well as diluted hydrofluoric acid. Suitable bases include, for example, sodium hydroxide, ammonia and carbonate as well as organic amines. Suitable catalysts in non-hydrolytic sol/gel processes include anhydrous halide compounds, for example BCl_3 , NH_3 , AlCl_3 , TiCl_3 or mixtures thereof.

In certain exemplary embodiments of the present invention, the sol may be further modified by the addition of at least one cross linking agent to the sol, the encapsulated active agent or the combination. The cross linking agent may comprise, for example, isocyanates, silanes, diols, di-carboxylic acids, (meth)acrylates, for example such as 2-hydroxyethyl methacrylate, propyltrimethoxysilane, 3-(trimethylsilyl)propyl methacrylate, isophorone diisocyanate, polyols, glycerine and the like. Biocompatible cross linkers such as glycerine, diethylene triamino isocyanate and 1,6-diisocyanato hexane may be preferably used.

Fillers can be used to modify the pore sizes and the degree of porosity, if desired. Some preferred fillers include inorganic metal salts, such as salts from alkaline and/or alkaline earth metals, preferably alkaline or alkaline earth metal carbonates, -sulfates, -sulfites, -nitrates, -nitrites, -phosphates, -phosphites, -halides, -

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sulfides, -oxides, as well as mixtures thereof. Further suitable fillers include organic metal salts, e.g. alkaline or alkaline earth and/or transition metal salts, such as formiates, acetates, propionates, malates, maleates, oxalates, tartrates, citrates, benzoates, salicylates, phthalates, stearates, phenolates, sulfonates, and amines as well
5 as mixtures thereof.

Preferably, porosity in the resultant bioactive material layers can be produced by treatment processes such as those described in German Patent publication DE 10335131 and in PCT Application No. PCT/EP04/00077. Further additives may include, e.g., drying-control chemical additives such as glycerol, DMF, DMSO or
10 any other suitable high boiling point or viscous liquids that can be suitable for controlling the conversion of the sols to gels and solid or semi-solid materials.

Sol-gels can be cured into a solid or semi-solid bioactive material layers. Curing into a gel, preferably an aerogel or xerogel, may be accomplished by, e.g., aging, raising of pH, evaporation of solvent or any other conventional method. The
15 sol may be preferably cured at room temperature, particularly where the materials used result in polymeric glassy composites, aerogels or xerogels.

Curing can be achieved by drying including a thermal treatment of the sol/combination or gel, in the range of about -200°C to $+200^{\circ}\text{C}$, such as in the range of about -100°C to 100°C , or in the range of about -50°C to 100°C , about 0°C to
20 90°C , or even from about 10°C to 80°C or at about room temperature. Drying or aging may also be performed at any of the above temperatures under reduced pressure or *in vacuo*.

Typically, many sol/gel derived materials obtained by hydrolysis reactions are biodegradable in physiologic fluids or cell culturing media themselves.
25 Additionally, biodegradable particles such as metals or polymers as described above may be embedded in sol-gel-derived materials in some exemplary embodiments, to provide the bioactive material layer. In such embodiments, degradation of the particles in the bioactive layer allows a partial degradation thereof, resulting in the loss of "anchoring sites" for the cells or tissue, thus leading to a detachment of the
30 tissue from the support.

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If particles are provided in the bioactive material layer, the particles can be selected from tubes, fibers, fibrous materials or wires or spherical or dendritic or any regular or irregular particle form and the particle sizes can be in, but not limited to, a range of about 1 nanometer (nm) up to about 1000 micrometer (μm), such as from 1
5 nm to 500 μm , or from 1 nm to 10 μm .. The degradable layer can be porous.

In further preferred embodiments, the support structure can be made from magnesium, magnesium compounds or magnesium alloys, and the bioactive layer comprises a slower degradation rate. In these embodiments, the bioactive layer including the cell culture or biological construct will be available after degradation of
10 the support structure for in-vivo implantation, wherein the layer is the subsequently degraded in-vivo.

In other embodiments, the support structure includes pH-sensitive or temperature sensitive polymers as described above, that change the properties upon change of pH or temperature either resulting, e.g., in a shift of surface charge,
15 volume and/or surface structure allowing the removal of the bioactive material layer together with the biological construct thereon *in toto*.

Functionalization and further exemplary embodiments

As described herein above, the bioactive material layer may include biodegradable inorganic, organic or inorganic-organic hybrid materials in a
20 particulate form. The materials can, e.g., comprise organic or inorganic micro- or nano-particles or any mixture thereof, which may also be included in the support structure. In the bioactive layer or in the support, the degradable material particles may be used to accelerate degradation and detachment of cells, in that selective degradation of the particles may mechanically destabilize the structure of the layer or
25 support structure.

Furthermore, it may also be preferable to add substantially non-degradable particles to the bioactive material layer or the support structure, regardless whether it already includes degradable particles or not.

For example, the particles added in some exemplary embodiments of the
30 present invention can include materials enhancing diagnostic properties or visibility

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in diagnostic methods, such as at least one of zero-valent metals, metal oxides, metal carbides, metal nitrides, metal oxynitrides, metal carbonitrides, metal oxycarbides, metal oxynitrides, metal oxycarbonitrides and the like. The particles may also be magnetic, e.g. to allow attracting, by magnetic forces, cell material or tissue adhering
5 to the magnetic particles floating in a medium after detachment from the support. Examples for magnetic particles are - without excluding others - iron, cobalt, nickel, manganese and mixtures thereof, for example iron, platinum mixtures or alloys, or for example, magnetic metal oxides such as iron oxide and ferrite.

Semi-conducting particles may also be used, e.g. to improve visibility for
10 diagnostic or monitoring purposes, for example semi-conductors, as mentioned as diagnostic agents in WO 2006/069677 and as described herein above.

Particles may also include super paramagnetic, ferromagnetic, ferromagnetic metal particles. Suitable examples are magnetic metals, alloys, preferably made of ferrites such as gamma-iron oxide, magnetite or cobalt-, nickel- or manganese
15 ferrites, particularly particles as disclosed in WO 83/03920, WO 83/01738, WO 85/02772 and WO 89/03675; and US patent 4,452,773, US patent 4,675,173; and WO 88/00060 and US patent 4,770,183; WO 90/01295 and WO 90/01899.

Additionally, particles incorporated into the support structure or in the bioactive material layer of the scaffolds may include carbon particles, for example
20 soot, lamp-black, flame soot, furnace soot, gaseous soot, carbon black, and the like, furthermore, carbon-containing nano particles and any mixtures thereof. Particle sizes especially for carbon-based particles are in the region of about 1 nm to 1,000 μm , such as from 1 nm to 300 μm , or from 1 nm to 6 μm .

Nano-morphous carbon species may also be used, such as fullerenes and the
25 like as mentioned herein above.

In a further exemplary embodiment, the particles can include polymers, oligomers or pre-polymeric particles such as beads. Examples of suitable polymers for use as particles in the present invention are mentioned herein above.

In certain exemplary embodiments, the particles can include electrically
30 conducting polymers, preferably from saturated or unsaturated polyparaphenylene-

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5 vinylene, polyparaphenylene, polyaniline, polythiophene, poly(ethylenedioxythiophene), polydialkylfluorene, polyazine, polyfurane, polypyrrole, polyselenophene, poly-p-phenylene sulfide, polyacetylene, monomers oligomers or polymers thereof or any combinations and mixtures thereof with other monomers, oligomers or polymers or copolymers made of the above-mentioned monomers. Particularly preferred are monomers, oligomers or polymers including one or several organic, for example, alkyl- or aryl-radicals and the like or inorganic radicals, such as, for example, silicon or germanium and the like, or any mixtures thereof. Preferred are conductive or semi-conductive polymers having an electrical resistance between $10\exp(-12)$ and $10\exp(12)$ Ohm·cm. It may particularly be preferred to select those polymers which comprise complexated metal salts.

In a further exemplary embodiment, cell attachment can be further promoted by a physical surface design of the bioactive material layer, hereinafter referred to as a "texture". Appropriate textures are ultra-micro porous or nano-porous structures. Nano-porous surfaces in a range of about 2 nm (nanometer) up to 20 nm particularly promote cell attachment. In exemplary embodiments, the attachment efficiency of cells may be tuned specifically by increasing or decreasing the size and density of the pores, depending on the properties of the cells or tissue to be grown on the scaffold. For example, for HUVEC (Human Umbilical Vein Endothelial Cells) it was found that the optimal attachment efficiency can be realized with a pore size at about 2 nm, for osteoblast cells it was found that the optimum pore size is at about 500 nm. Applicable pore sizes for improving cell attachment in exemplary embodiments may therefore be selected in a range from about 2 nm to about 10 μ m (micrometer), depending on the cell type and cell entity. It is assumed that the implementation of the aforesaid textures is mimicking the known tight and gap junctions of cell-cell contacts. These textures may be applied to all materials and material combinations as described herein.

In exemplary embodiments it can be preferred to have, as the bioactive material layer or as an additional layer on top thereof, a degradable biochemical active surface on the scaffold. Biochemical active surfaces are capable to interact

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with glycoproteins, carbohydrates, proteins, lipids, lipoproteins, glycolipids and similar compounds that allow cell attachment, such as membrane or transmembrane receptors. Examples for cell adhesion proteins include selectins, integrins as well as cadhereins. Those membrane compounds of cells are capable to bind to absorptive
5 surfaces or physiologic ions. Presence of calcium, sulfur, magnesium or heavy metals, preferably as ions, on the surface of scaffolds, can promote the adhesion of such cell attachment structures of cells, whereby the selection of the ions and ions entities significantly allows attracting or attaching selectively specific cells and cell types. An isolated specific membrane receptor or transmembrane receptor can be
10 included as a biochemical active surface in the bioactive material layer. The bioactive material layer may comprise at least one of physiologically acceptable ions and at least one isolated specific membrane receptor or transmembrane receptor, or both.

According to a further exemplary embodiment of the present invention,
15 physiologic ions or salts may be embedded into the surface of the bioactive material layer and/or into the support structure material. Preferably, the material may include at least one physiologically acceptable ion, i.e. cation or anion, that is embedded into the material structure, e.g. at the surface. Preferably, the ions are selected from alkaline or alkaline earth ions such as calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium
20 (Na^{+}), potassium (K^{+}), lithium (Li^{+}), cesium (Cs^{+}), aluminum (Al^{3+}) a transition metal ion such as Zn^{2+} , Ag^{+} , a heavy metal ion, anions such as sulfur, Chloride(Cl^{-}), nitrate (NO_3^{-}), sulfate (SO_4^{2-}), phosphate (PO_4^{3-}) etc, most preferred ions of Ca and S and Mg.

Such ions may include any ion having a beneficial effect on the tissue grown.
25 The ratio between some of the anions and cations can be varied, according to the underlying nature of the cell type that selectively shall be attached. A mixture can be selected resulting in different patterns of ions and ion concentrations. As it is widely accepted, the attachment of cells to physiologic matrices such as extracellular matrix (ECM) is depending on the interaction between cell adhesion molecules (CAMs),
30 such as cadherins, Ig superfamily of CAMs (for example N-CAM, V-CAM) and

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integrins. The ECM, for, example is well known to comprise substances such as proteoglykanes that are chemically containing a core protein and glykosaminoglykanes. Like ceratane sulphate or heparansulfate, many of those compounds comprise sulfur groups and the like, e. g., or they have partially the
5 function to bind selectively physiologic ions such as, sodium, magnesium or calcium. Incorporating a tissue-specific composition of ions within the support material or the bioactive material layer may directly correlate with the selective attachment and growth of cells.

Conventional techniques may be used for measuring or verifying the surface
10 structure and composition. One exemplary method is ESCA (Electron Spectroscopy for Chemical Application), another suitable method is EDX (Energy Dispersive X-ray Spectroscopy). The enrichment or doping of the material with at least one physiologic ion results preferably in following content (atom%): 0,1% up to 90%, preferably 1% to 85%, most preferred 5% to 25%, as measured by EDX.

15 Ion exchangers which may be included in the scaffolds include those for binding positively charged ions or cations, which display on their surface negatively charged groups; or those for binding negatively charged ions or anions, which display on their surface positively charged groups. The ion exchanger can be composed of the solid support material, a liquid or gel, or any combination thereof,
20 such as for example a hydrogel or polymer composed for easily hydrated groups such as cellulose consisting of polymers of sugar molecules. These materials consist of polymeric matrixes to which are attached functional groups. The chemistry of the matrix structure is polystyrenic, polyacrylic or phenol-formaldehyde. The functional groups are numerous: sulfonic, carboxylic acids, quaternary, tertiary and secondary
25 ammonium, chelating (thiol, iminodiacetic, aminophosphonic and the like). The various types of matrices and their degree of cross linking translate into different selectivity for given species and into different mechanical and osmotic stability. Ion exchange resins are also characterized by their operating capacities function of the process conditions. Ion exchange resins are mostly available in a moist beads form
30 (granular or powdered forms are also sometime used, dry form is also available for

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applications in a solvent media) with a particle size distribution typically ranging 0.3 – 1.2 mm (16 – 50 mesh) with a gel or macro porous structure. The ion exchanger may be included in at least one part of the scaffold, for example in the active layer or in the structural material. Other useful materials are absorbents to absorb at least one
5 compound of the cells, organized cells, tissue, organ or biologic construct, either in-vivo or ex-vivo.. Suitable absorbers, for example, are used to absorb proteins. For protein absorption diethylaminoethyl (DEAE) or carboxymethyl (CM) absorbers are appropriate. Since proteins are charged molecules, proteins in the cultivation system will interact with the absorber depending on the distribution of charged molecules on
10 the surface of the protein, displacing mobile counter ions that are bound to the resin. The way that a protein interacts with the absorber material depends on its overall charge and on the distribution of that charge over the protein surface. The net charge on a given protein can depend on the composition of amino acids in the protein and on the pH of the fluid. The charge distribution can further depend on how the charges
15 are distributed on the folded protein. An appropriate absorber or combination of absorbers and/or the pH of the fluid can be selected based on the protein's isoelectric point, for adjusting the absorption properties and function.

In addition to conventional absorbents, further useful absorbents can include from materials that comprise imidazolium, quaternary ammonium, pyrrolidinium,
20 pyridinium, or tetra alkylphosphonium as the base for the cation, whereby possible anions include hexafluorophosphate $[\text{PF}_6]^-$, tetrafluoroborate $[\text{BF}_4]^-$, bis(trifluoromethylsulfonyl) imide $[(\text{CF}_3\text{SO}_2)_2\text{N}]^-$, triflate $[\text{CF}_3\text{SO}_3]^-$, acetate $[\text{CH}_3\text{CO}_2]^-$, trifluoroacetate $[\text{CF}_3\text{CO}_2]^-$, nitrate $[\text{NO}_3]^-$, chloride $[\text{Cl}]^-$, bromide $[\text{Br}]^-$, or iodide $[\text{I}]^-$, among many others. Any combination of a absorbing material can be
25 selected. Suitable absorbers are also activated carbon or activated carbon-like materials, chelating agents such as penicillamine, methylene tetramine dihydrochloride, ethylenediaminetetraacetic acid (EDTA), Distearyl dimethylamine (DMSA) or deferoxamine mesylate and the like. The absorber can be provided as a liquid solution, gel, solid or any combination thereof. The solid can include particles,

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a structured mold or any combination thereof. The absorber can be included in the active layer and/or the structural material of the scaffold.

Further, beneficial agents may be added to the bioactive material layer and/or the support structure. Beneficial agents can be selected from biologically active agents, pharmacological active agents, therapeutically active agents, diagnostic agents or absorptive agents or any mixture thereof. Beneficial agents can be incorporated partially or completely into the support or bioactive layer or into a further overcoating of the scaffold. Furthermore, it is also one aspect of the present invention to optionally or further coat the inventive scaffold with beneficial agents partially or completely, e.g. with growth factors, gene-vectors etc.

Examples of beneficial ingredients include biologically, therapeutically or pharmacologically active agents such as drugs or medicaments, diagnostic agents such as markers, ion exchangers or absorptive agents. The active ingredients may be incorporated into the scaffold or being coated on at least a part of the scaffold. Biologically or therapeutically active agents comprise substances being capable of providing a direct or indirect therapeutic, physiologic and/or pharmacologic effect in a cell, tissue or a human or animal organism. A therapeutically active agent may include a drug, pro-drug or even a targeting group or a drug comprising a targeting group. An "active ingredient" may further include a material or substance which may be activated physically, e.g. by radiation, or chemically, e.g. by metabolic processes.

Suitable therapeutically active agents may be selected from the group of enzyme inhibitors, hormones, cytokines, growth factors, receptor ligands, antibodies, antigens, ion binding agents such as crown ethers and chelating compounds, substantial complementary nucleic acids, nucleic acid binding proteins including transcriptions factors, toxins and the like. Further examples of active agents, beneficial agents, absorbents, signal-generating agents and diagnostic agents or markers are disclosed in WO 2006/069677, all of which are incorporated herein by reference.

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Geometric scaffold structures

The support structure may have any desired shape or form, depending on the specific application, suitable for growing cells or tissue on it. For example, the support structure can have a honeycomb, mesh or tubular structure for ex-vivo cell
5 culturing systems, or it may be in the form of a "must-fit" implant for replacement of bone or cartilage, which may be implanted into the human or animal body after tissue has been grown on the scaffold in an ex-vivo culturing system. Basically, the scaffold can be made from one part or from an assembly of multiple parts as desired.

Material used to make the support and/or the bioactive material layer may be
10 porous, e.g. nanoporous, ultramicroporous, microporous or mesoporous or macroporous, or may have combined pores or porosities. The materials can be completely or partially porous within any section or part or at different sections or parts. The porous scaffold material can include a gradient of different porous layers or sections in any desired geometric or three-dimensional direction. For example, the
15 bioactive material layer can have a bulk volume porosity of about 10-90 %, such as from about 30% to 80%, or from about 50% to 80%. Also, such pores can have a dimension suitable for osteoconduction, such as from about 50 to 1000 μm . Porosity and average pore sizes may be measured by conventional methods such as adsorptive methods, e.g. N_2 - or Hg- porosity measurements. The porous structure can also be
20 partially or completely a mesh-like porous structure or a lattice, or it may comprise a mesh-like trabecular, regular or irregular or random or pseudo-random structure, or any combination thereof.

In an exemplary embodiment, the scaffold can be provided in a shape of a cylinder having at least one excavation. For example, the excavation may pass
25 through a mould body connecting one side of the surface with another side. The excavation may include a flow-channel for inflow or outflow or through-flow of a fluid, fluid mixture or components or compounds of a fluid or fluid mixture, wherein the flow-channel can be centric or eccentric, linear or non-linear. The scaffold may include a Y-like or star-shaped form, at least in one plane, whereby the form has at
30 least three parts that intersect at a node and the parts may be formed to lamellas with

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a linear profile in the cross-section. The combined shape can be symmetric or asymmetric, regular or irregular, whereby each individual lamella can have a different geometry.

The scaffold may also have a honeycomb-like structure. The honeycomb
5 configuration can be provided, e. g., as a pentagonal, hexagonal, polygonal or tubular or rectangular or any other geometric configuration, preferably a symmetric pattern.

Special embodiments

In an exemplary embodiment, the bioactive material layer includes a combination of degradable metal particles, e.g. particles based on Mg, Ca, and/or Zn
10 or alloys thereof, as described above, with magnetic particles, e.g. iron-based particles, both optionally embedded in a degradable or non-degradable polymer or sol/gel-derived material.

In a further exemplary embodiment having enhanced biocompatibility or haemocompatibility, the bioactive material layer includes nanomorphous carbon
15 species, such as fullerenes, for example, C36, C60, C70, C76, C80, C86, C112 etc., or any mixtures thereof, furthermore, nanotubes such as MWNT, SWNT, DWNT, random-oriented Nanotubes, as well as so-called fullerene onions or metallo-fullerenes, in a silane based inorganic-organic hybrid material, which may optionally be prepared by using sol/gel technology.

20 Completely degradable scaffolds may provide a degradation rate that corresponds to the re-growth or repair rate of the tissue. Typical biodegradation rates for maintaining the structure or structural integrity of a scaffold can be, e. g., about 4-10 weeks for cartilage repair and about 3-8 weeks for bone repair. The mechanical requirements of the scaffolds can be highly dependent on the type of tissue being
25 replaced. For example, cortical bone has a Youngs Modulus of 15-30 GPa whereby cancellous (or spongy, trabecular) bone has a Youngs Modulus of 0.01-2GPa. Cartilage has a Youngs Modulus of less than 0.001 GPa. The materials used for a scaffold in any particular case should be selected appropriately to reflect such considerations.

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Manufacturing

The scaffolds can, for example, be manufactured using conventional methods, for example by coating, sputtering, molding or metallizing a template as disclosed in US-Patent Applications 12/016,835; 12/030,304; 12/016,519; 12/030,392;
5 12/030,350; 12/030,315; 12/030,680; 12/098,282; 12/033,238 and 12/016,536. Other suitable methods may use an organic precursor or polymer or pre-polymer and carbonize the material. Such techniques are described, e. g., in WO 2005/021462; WO 2004/082810; WO 2004/101177; WO 2004/101017; WO 2004/105826; WO 2004/101433; WO 2005/012504; WO 2005/042045; WO 2005/065843; WO
10 2006/074809; and WO 2006/069677

Techniques for producing porous support structures are disclosed in WO2005/021462 A1, including techniques for introducing porosity into carbon materials produced by carbonization of organic polymer precursors. The aforesaid techniques also permit molding of a scaffold to any desired geometric shape.
15 Production of sol/gel-based coatings that can be degraded in physiologic fluids and which may be applied to at least a portion of the scaffold, are described, e. g., in WO 2006/077256 or WO 2006/082221.

Tissue engineering method

In an exemplary embodiment of the present invention a method for tissue
20 engineering is provided, comprising the steps of providing, in a cell culture system or bioreactor, a scaffold as described herein, the scaffold comprising a support structure having an outer surface, at least a part of the outer surface being covered with a bioactive material layer that allows cell attachment, wherein the bioactive material is at least partially degradable in an environment of use to allow a detachment of the
25 cells from the support structure by degradation of the bioactive material layer; inoculating the scaffold with cells or living tissue; cultivating the inoculated scaffold in a suitable environment to grow tissue; harvesting the grown tissue after degradation of the bioactive layer.

The properties of the scaffold or the bioactive material layer may be modified
30 as described above, e.g. with other substances selected from organic and inorganic

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substances or compounds. Examples include compounds or ions of iron, cobalt, copper, zinc, manganese, potassium, magnesium, calcium, sulphur or phosphorus. The incorporation of such additional compounds may be used, for example, to promote the growth of certain tissue or cells on the scaffold. Further, impregnation or coating of the scaffold with carbohydrates, lipids, purines, pyrimidines, pyrimidines, vitamins, proteins, growth factors, amino acids and/or sulfur sources or nitrogen sources are also suitable in promoting growth. The following substances may also be used to stimulate cell growth: bisphosphonates (e.g., risedronates, pamidronates, ibandronates, zoledronic acid, clodronic acid, etidronic acid, alendronic acid, tiludronic acid), fluoride (disodium fluorophosphate, sodium fluoride); calcitonin, dihydrotachystyrene as well as all growth factors and cytokins (epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factors (FGFs), transforming growth factors b (TGFs-b), trans-forming 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 (IL-6), interleukin 8 (IL-8), tumor necrosis factor a (TNF-a), tumor necrosis factor b (TNF-b), interferon g (INF-g), monocyte chemo-tactic protein, fibroblast stimulating factor 1, histamine, fibrin or fibrinogen, endothelin 1, angiotensin II, collagens, bromocriptine, methysergide, methotrexate, carbon tetrachloride, thioacetamide, ethanol.

In an exemplary embodiment of the present invention, the scaffold is loaded with viable and/or propagable biological material, capable of replication, such as single-cell or multi-cell micro organisms, fungi, spores, viruses, plant cells, cells culture or tissue or animal or human cells, cell cultures or tissue or mixtures thereof. Such loading typically leads to extensive immobilization of the biological material.

The loading can be performed with tissue-forming or non-tissue-forming mammalian cells, primary cell cultures such as eukaryotic tissue, e.g., bone, cartilage, skin, liver, kidney as well as exogenous, allogenic, syngenic or autologous cells and cell types and optionally also genetically modified cell lines and in particular also nerve tissue.

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The biological material can be applied to the scaffold using conventional methods. Examples include, e. g., immersion of the scaffold in a solution/suspension of the cell material, spraying the scaffold with cell material solution or suspension, inoculating a fluid medium in contact with the scaffold and the like. An incubation
5 time may be used after loading to allow the immobilized biological material to completely permeate the supporting body or substrate.

Such scaffolds are suitable in particular for immobilizing and propagating all types of tissue cultures, especially cell tissues. In such processes, the cells can be supplied with liquid or gaseous nutrients in a bioreactor, while metabolites may be
10 removed easily with a fluid flow.

The scaffolds, optionally installed in suitable housings to form cartridge systems which are optionally loaded with different cell cultures, may be immersed in a single culture medium for the sake of reproduction and may be removed from the culture medium after a certain period of time, when the bioactive material layer is
15 sufficiently degraded to separate the tissue from the support structure.

Suitable ex-vivo bioreactors include e.g., flasks, bottles, in particular cell culture flasks, roller bottles, spinner bottles, culture tubes, cell culture chambers, cell culture dishes, culture plates, pipette caps, snap cover dishes, cryotubes, agitated reactors, fixed bed reactors, tubular reactors and the like.

20 Before, during or after loading with the biological material, the scaffold is brought in contact with a fluid medium. The fluid medium may optionally be a different medium before loading than after loading. The term "fluid medium" includes any fluid, gaseous, solid or liquid, such as water, organic solvents, inorganic solvents, supercritical gases, conventional substrate gases, solutions or suspensions
25 of solid or gaseous substances, emulsions and the like. The medium is preferably selected from liquids or gases, solvents, water, gaseous or liquid or solid reaction educts and/or products, liquid culture media for enzymes, cells and tissues, mixtures thereof and the like.

Examples of liquid culture media include, for example, RPMI 1640 from Cell
30 Concepts, PFHM II, hybridoma SFM and/or CD hybridoma from GIBCO, etc. These

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may be used with or without serum, e.g., fetal bovine serum medium with or without amino acids such as L-glutamine. The fluid medium may also be mixed with biological material, e.g., for inoculating the scaffold.

5 The contact may be accomplished by complete or partial immersion of the scaffold or the housing/container holding it into the fluid medium. The scaffolds may also be secured in suitable reactors so that fluid medium can flow through them. An important criterion here may be the wettability and removability of any enclosed air bubbles from the substrate material. Evacuation, degassing and/or flushing operations may be necessary here and may be used as needed.

10 The scaffold can be immersed in a solution, emulsion or suspension containing the biological material for a period for time from about 1 second up to about 1000 days or may be inoculated with it, optionally under sterile conditions, to give the material an opportunity to diffuse into the porous body and form colonies there. The inoculation may also be performed by spray methods or the like.

15 The fluid medium, e.g., a culture medium, may be moved or agitated to ensure the most homogeneous possible vital environment and supply of nutrients to the cells. This may be accomplished through various methods, e.g., by moving the scaffold in the medium or moving the medium through the scaffold. This is usually done for a sufficient period of time to permit growth, reproduction or adequate
20 metabolic activity of the biological material.

Harvesting of tissue or cells may be done as desired and as further described herein, after degradation of at least a part of the bioactive material layer.

In an exemplary embodiment of the present invention, the scaffold may also be used in ex-vivo perfused systems, such as assisted systems, to partially or
25 completely replace organ functions. For example the scaffold can include supporting structure formed from a carbon-based material, preferably made from pyrolytic, glassy carbon, diamond-like carbon or any other carbon material obtainable by carbonization or using CVD or PVD methods, for example a porous, spongy or trabecular carbon body, and a bioactive surface structure that supports cell
30 attachment. The bioactive surface structure can include a biodegradable polymer

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coating such as a poly(lactic acid) coating, or other such polymers as described herein above. For long-term cultivation, the bioactive material layer comprises a bioactive nano-structure with a porosity in a range of 2nm up to 500nm to support cell attachment of hepatocytes or any co-culture comprising hepatocytes or hepatic progenitor cells. Preferably, the scaffold has a random or pseudo-random or geometric mesh-structure in order to trigger a trabecular growth pattern of the adherent cell culture and to increase the available biologically active surface of the scaffold within the assisted system.

Hence, the scaffold can have any desired superordinate mould structure, preferably with flow-channels or appropriate shape for gas exchange within a compartment of the scaffold. In some preferred configurations, the compartment in the scaffold is first used to seed, for example, hepatocytes or hepatic progenitor cells or any co-culture comprising hepatocytes or hepatic progenitor cells, and to increase the cell density until the complete surface of the scaffold has been covered by the culture. Such a pre-grown tissue on the scaffold can subsequently be used as a part or component of an liver assisting system, taking over at least a part of the organ function of liver tissue outside the patient's body. In this embodiment patient blood is circulated through the compartment of the scaffold comprising the liver tissue with sufficient contact time.

In other embodiments, the scaffold can be made from a carbon hollow-fiber membrane and the bioactive material layer is comprised by a biodegradable polymer coating such as a PLA coating. The layer can provide a nano-structured porous surface for promoting attachment and growth of, e.g. different entities of renal cells, renal progenitor cells or any combination thereof, preferably comprising renal proximal tubule cells or the like. An exemplary use of this scaffold includes a first step of seeding and cultivating the cells on the scaffold and subsequently providing the scaffold and respective compartment to a kidney assisting system.

In further embodiments the scaffolds include magnesium-based alloys as the bioactive material layer. Such scaffolds can preferably be used to seed and cultivate

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chondroblasts, chondrocytes, osteoblasts or osteocytes or any co-culture thereof within a bio-reactor to produce cartilage or bone tissue.

* * *

Having thus described in detail several exemplary embodiments of the
5 present invention, it is to be understood that the invention described above is not to be limited to particular details set forth in the above description, as many apparent variations thereof are possible without departing from the spirit or scope of the present invention. The embodiments of the present invention are disclosed herein or are obvious from and encompassed by the detailed description. The detailed
10 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 Figures.

The foregoing applications, and all documents cited therein or during their prosecution (“appln. cited documents”) and all documents cited or referenced in the
15 appln. cited documents, and all documents cited or referenced herein (“herein cited documents”), and all documents cited or referenced in the herein cited documents, together with any manufacturer’s instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and
20 may be employed in the practice of the invention.

The invention is further characterized by the following claims, which should not be construed to limit the invention to the feature combinations given therein.

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Claims:

1. A scaffold for tissue engineering, comprising:
a support structure having an outer surface, at least a part of the outer surface
5 being covered with a bioactive material layer that allows cell attachment, wherein the
bioactive material is at least partially degradable in an environment of use to allow a
detachment of the cells from the support structure by degradation of the bioactive
material layer.
- 10 2. The scaffold of claim 1, wherein the environment of use is a liquid medium
in a cell culture vessel or bioreactor or a body fluid.
3. The scaffold of any one of claims 1 or, wherein the bioactive material layer
comprises a texture or morphology that promotes cell attachment, and is preferably
15 mimicking the tight and gap junctions of cell-cell contacts of the tissue to be grown
on the scaffold.
4. The scaffold of any one of claims 1 to 3, wherein the bioactive material
layer is porous, and wherein the pores have an average pore size from about 2 nm to
20 about 10 μm .
5. The scaffold of claim 4, wherein the average pore size is from about 2 nm
to about 500 nm.
- 25 6. The scaffold of any one of claims 1 to 5, wherein the bioactive material
layer has a bulk volume porosity of about 10-90 %, or from about 30% to 80%, or
from about 50% to 80%.
7. The scaffold of any one of claims 1 to 4 or 6, wherein the pores have a
30 dimension suitable for osteoconduction, such as from about 50 to 1000 μm .

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8. The scaffold of any one of claims 1 to 7, wherein the bioactive material layer is configured to interact with at least one of glycoproteins, carbohydrates, proteins, lipids, lipoproteins, glycolipids, selectins, integrins, cadhereins, membrane
5 or transmembrane receptors, or similar compounds that promote cell attachment.

9. The scaffold of any one of claims 1 to 8, wherein the bioactive material layer comprises physiologically active salts or ions, such as ions of calcium, sulfur, magnesium, a heavy metal, sodium, potassium, phosphate, sulfate, chloride or
10 nitrate.

10. The scaffold of any one of claims 1 to 9, wherein the bioactive material layer comprises an inorganic material or consists of an inorganic material.

15 11. The scaffold of claim 10, wherein the bioactive material layer includes at least one of a biodegradable metal or a metal alloy.

12. The scaffold of claim 11, wherein the at least one of a biodegradable metal or metal alloy includes at least one of an alkaline metal, an alkaline earth
20 metal, Fe, Zn, Al, Mg, Ca, Zn, W, Ln, Si, or Y.

13. The scaffold of any one of claims 11 or 12, wherein the bioactive material layer includes a magnesium alloy comprising more than about 90 % of Mg, between about 4-5 % of Y, and between about 1.5-4 % of other rare earth metals.

25

14. The scaffold of any one of claims 11 to 13, wherein the bioactive material layer includes the at least one of a biodegradable metal or metal alloy in a particulate form.

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15. The scaffold of claim 14, wherein the at least one of a biodegradable metal or metal alloy in a particulate form is combined in the bioactive material layer with at least one of metallic particles made of a more noble metal to provide a corrodible bioactive material layer, or with at least one magnetic material particle.

5

16. The scaffold of any one of claims 1 to 9, wherein the bioactive material layer comprises an organic material or consists of an organic material.

17. The scaffold of claim 16, wherein the organic material is completely biodegradable.

18. The scaffold of any one of claims 1 to 9, wherein the bioactive material layer includes or consists of an inorganic-organic hybrid material.

19. The scaffold of claim 18, wherein the hybrid material is provided using a sol-gel processing technique.

20. The scaffold of any one of claims 18 or 19, wherein the bioactive material layer includes a biodegradable inorganic-organic hybrid material provided in a particulate form.

21. The scaffold of any one of claims 1 to 20, wherein the bioactive material layer is completely degradable.

22. The scaffold of any one of claims 1 to 21, wherein the support structure material and the bioactive material layer are both degradable.

23. The scaffold of claim 22, wherein the degradation rates of the support structure material and the bioactive material layer are different.

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24. The scaffold of claim 21, wherein the support structure is made of a substantially non-degradable material.

25. The scaffold of any one of claims 1 to 24, wherein the support structure is
5 made from at least one of an inorganic material, an organic material, an inorganic-organic hybrid material, a carbon material, a polymer material, a ceramic material, a metal or metal alloy material, or a composite or combinations thereof.

26. The scaffold of any one of claims 1 to 25, further comprising, in the
10 support or in the bioactive material layer, at least one additive selected from at least one of an inorganic filler or an organic filler, a salt, hydroxyl apatite; or a beneficial agent.

27. The scaffold of claim 26, wherein the beneficial agent includes at least
15 one of a pharmacologically, therapeutically, biologically or diagnostically active agent, or an absorptive agent.

28. A use of a scaffold of any one of claims 1 to 27, for tissue
engineering.
20

29. A use of a scaffold of any one of claims 1 to 28, for use in ex-vivo perfused systems, such as assisted systems, to partially or completely replace organ functions.

25 30. A use of an at least partially degradable bioactive material layer on the surface of a support structure of a scaffold for tissue engineering, for facilitating separation or detachment of tissue grown on the scaffold by degrading the bioactive material layer in an environment of use.

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31. The use of claim 30, wherein the degrading of the bioactive material layer is induced by a stimulus, such as an electrical current or voltage, or by a change in the pH of the environment of use.

5 32. A method for tissue engineering, comprising the following steps:

- providing, in a cell culture system or bioreactor, a scaffold as described in any one of claims 1 to 27, the scaffold comprising a support structure having an outer surface, at least a part of the outer surface being covered with a
10 bioactive material layer coating that allows cell attachment, wherein the bioactive material is at least partially degradable in an environment of use to allow a detachment of the cells from the support structure by degradation of the bioactive material layer;
- inoculating the scaffold with cells or living tissue;
- 15 - cultivating the inoculated scaffold in a suitable environment to grow tissue;
- harvesting the grown tissue after degradation of the bioactive layer.

20 33. The method of claim 32, wherein the degradation of the bioactive material layer is induced by a stimulus, such as an electrical current or voltage, or by a change in the pH of the environment of use.

25