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(54) **Title:** TISSUE REGENERATION BY ENDOCULTIVATION

(57) **Abstract:** A method for tissue regeneration for a patient includes the steps of providing a scaffold for the replacement tissue and coating and/or inoculating the scaffold with cells and/or growth/differentiating/morphogenetic factors(cytokines) capable of forming or inducing formation of the replacement tissue. The scaffold is implanted into fat or muscle tissue in a host and the scaffold, replacement tissue and a portion of the blood supply of the replacement tissue is harvested when sufficient tissue formation and angiogenesis of the replacement tissue has occurred. The scaffold, replacement tissue and blood supply is transplanted or translocated to a site where the replacement tissue is required and at least part of a blood supply of the replacement tissue is reconnected to a blood vessel at the site.

TITLE

"TISSUE REGENERATION BY ENDOCULTIVATION"

The present invention relates to replacement tissue, and in particular, to in vivo growth of replacement tissue suitable for use in tissue grafting or organ endocultivation procedures in a patient, and to scaffolds and methods for growing said replacement tissue in vivo.

BACKGROUND OF THE INVENTION

A vast array of diseases exist where organ tissue is damaged and replacement tissue is required in order to restore or improve the functioning of a patient's own organ. Furthermore, tissue loss or failure of organ tissue to develop normally can also occur as a consequence of physical trauma or congenital abnormalities.

An example of a condition where tissue is irreparably damaged is congestive heart failure, the ineffective pumping of the heart caused by the loss or dysfunction of heart muscle cells, which afflicts 4.8 million people, with 400,000 new cases each year in the United States alone. One of the major contributors to the development of this condition is a myocardial infarction. Impairments of the heart and circulatory system represent a major cause of death and disability in most developed countries of the world. As a terminally differentiated tissue, cardiac tissue does not self-renew, and thus once cardiac myocytes are damaged, they have no capacity to repair. Once a patient suffers a significant degree of damage to the myocardium, one of the few remaining options is a transplant from a heart donor.

Another example is the liver which may receive impairment through toxins, infection, trauma, cancer or autoimmune diseases. Liver tissue has a potential to regenerate which makes it also interesting for in vivo tissue engineering procedures.

Especially tissue engineering procedures that deliver a vessel pedicle for the engineered tissue are suitable for transplantation of the said tissue into the liver/bowl blood circuit. Therefore, in vivo tissue engineering procedures with endocultivation of liver cells plus private blood vessel supply could replace the existing liver transplantation system requiring liver donors..

Damaged coronary arteries are also a problematic tissue to replace. Generally, a blood vessel from another part of a patient's body is harvested and grafted to replace the damaged or blocked coronary artery. However, the vessels usually available from the patient are veins, which are about one-third the thickness of arteries. Veins are not designed to meet the high-pressure demands of arteries, and as such, they cannot take the stress of acting as a replacement artery indefinitely.

Musculo-skeletal diseases such as arthritis, back pain and bone loss are particularly problematic, as otherwise healthy people are generally in chronic pain and unable to work. While arthritis can be cured with artificial joint replacements, these implants have a finite life span. Revision surgery is required and the main problem with this type of surgery is bone loss.

While it is possible to use tissue from a donor to replace tissue that is lost or damaged in another patient, there are significant problems associated with the phenomenon of tissue rejection. In traditional organ transplants, rejection occurs when the host body rejects the transplanted organ. In bone marrow transplants however, rejection actually causes the immune system to turn against the host body. This effect is known as graft versus host disease (GVHD). Furthermore, organ transplantation is limited in most situations due to a lack of donor tissue, such that patient need exceeds organ availability.

Various attempts have been made to grow tissues in the laboratory. However, most attempts at this type of tissue growth have failed, or are severely limited by the slow rate at which tissue can be grown. Furthermore, the growth of three-dimensional tissues (ie. organs or parts thereof) has, to date, been grossly unsuccessful.

Most Europeans will suffer from arthritis or back pain, which keeps them off work. 103 Million Europeans need an artificial replacement joint to regain their quality of life. These replacements are made of solid titanium or stainless steel and have a finite life span, so that revision surgery is mandatory. New inventions like Minimal Invasive Surgery computer navigation or robotic assistance for hip replacements have improved joint replacement surgery only slightly.

This application includes a process to grow an individually shaped Joint-Like-Structure in the Latissimus dorsi muscle of patients with arthritis, which can later be transplanted to replace the original joint. The goal is to use the patient as the bioreactor to prevent the common problems of engineering tissue in vitro in the laboratory. The same technique could be used to grow more complex organs in the future. Our vision is to develop a procedure where patients cultivate their own organs, a move away from biotechnology towards "tissue endocultivation".

Interest amongst scientists and patients in tissue regeneration has grown. This technology may eventually allow us to produce substitute organs or body parts inside our own human bodies. Success may make the search for organ donors and the well-described problems associated with allogenic organ transplants redundant.

Tissue engineering, growing tissues and organs *in vitro* in the laboratory, is now a common approach in attempts to grow new tissues and organs. A main advantage of this approach is control over growth sequences due to manipulation and constant supply of growth factors. A major disadvantage is the lack of functional loading, missing blood supply for transplantation and possible host rejection of the tissue. In vitro engineering cannot produce big complex structures yet, which can be transplanted into the patient, because of problems with nutrition.

These disadvantages disappear when the patient cultivates the tissue or organ in his own body. An example of such patient-based tissue cultivation is Autologous Chondrocyte Implantation (ACI), a cell therapy technique to repair local defects in cartilage, pioneered in Sweden and now routinely used in several European centres. A major step forward in patient-based tissue cultivation was seen in 2004, it was demonstrated in a pioneering *in vivo* engineering technique how a patient can cultivate a new individually shaped autologous mandible in his own back to reconstruct his resected original mandible. (Lancet 2004).

Osteoarthritis is not a disease of a single tissue, but of a complete organ: the joint. A clear step beyond the state-of-the-art will be for patients with arthritis to cultivate an individually shaped Joint-Like-Structure (JLS) in their back, which requires the combination of bone, cartilage, fibrous tissue and vessels for blood supply. This JLS can later be transplanted to replace the damaged original joint.

Accordingly, there is a need for improved tissue regeneration methods.

SUMMARY OF THE INVENTION

The present invention is predicated in part on the finding that replacement tissue may be grown in vivo in certain tissues of the body, and be provided with a blood supply via angiogenesis of the replacement tissue and existing vessel pedicles at the cultivation/donor site. These blood vessels may thus be sutured to the blood vessels that vascularise the site where the replacement tissue is required. Alternatively, the replacement tissue may simply be translocated to the site where the replacement tissue is required, which is close to where the replacement tissue has grown in vivo, thus negating the need for suturing of blood vessels that have vascularised the replacement tissue. The method is special as the patient will be his own bioreactor cultivating autologous tissue inside his own body (endocultivation) for regeneration and replacement of impaired or missing tissue (defect). For endocultivation the required scaffolds, cells and growth factors/cytokines/morphogenetic proteins will be inserted into the patient. It is optional to start cultivation outside the patient in in vitro bioreactors prior to implantation in the in vivo bioreactor of the patient. With this technique it will be possible to endocultivate all kinds of tissue allowing for ingrowth of a blood supply. Thus, this technique will allow for growth of large tissue quantities and for subsequent transplantation or translocation after cultivation period. Additionally, the combination of various tissue for example bone and cartilage or liver cells and other liver organ tissue is possible. Following this, complex structures such as joints, liver organs, hearts, kidneys or other human organs can be grown.

Thus, in a first broad form, the present invention relates to a method for tissue regeneration for a patient, said method comprising:

a) providing a scaffold for the replacement tissue;

b) coating and/or inoculating the scaffold with cells and/or growth/differentiating/morphogenetic factors(cytokines) capable of forming or inducing formation of the replacement tissue;

c) implanting the scaffold subcutaneously on contact to a fascia, below the pereosteum, or into fat or muscle tissue in a host;

d) harvesting the scaffold, replacement tissue and a portion of the blood supply of the replacement tissue when sufficient tissue formation and angiogenesis of the replacement tissue has occurred;

e) transplanting or translocation of the scaffold, replacement tissue and the blood supply of the replacement tissue to a site where the replacement tissue is required in the patient; and

f) connecting at least part of a blood supply of the replacement tissue to a blood vessel at the site where the replacement tissue is required (microsurgery and anastomoses techniques)

Preferably, the scaffold is moulded to a given size and shape, said size and shape being substantially the same as the site where the replacement tissue is required.

The shape and contour should be an exact copy of normal anatomy to be replaced. For example: Joint contour and shape will be determined using 3 dimensional CT /MRI scan of the relevant joint. Using our previous experience a 3D replica or replacement of this structure will be made with the CT scan data (Warnke et al. Lancet. 2004, Aug 28;364(9436):766-70)). The perfect individual replacement will be designed on the screen using CAD and virtually transplanted to check for exact fit. Anatomical

structures like nerve or vessel canals can be inserted into the replacement. This data will be forward to computer assisted manufacturing processes.

Different technologies can be assessed to ascertain the best one for our needs such as 1. Selective Laser Melting, 2. Rapid Prototyping / Micro-assembly and 3. Laminated Material Design.

Even more preferably, the cells capable of forming the replacement tissue are selected from osteoblasts, osteoblast precursor cells, skeletal muscle precursors (myoblasts), periosteal cells and periosteal stem cells, cardiac and endothelial progenitor cells, nasal stem cells, stromal cells, neuronal stem cells, chondrocytes, multipotent adult progenitor cells, peripheral blood stem cells, embryonic carcinoma cells isolated from teratocarcinomas, liver stem cells, pancreatic stem cells, corneal limbal stem cells, mammary stem cells, salivary gland stem cells and dermal hair follicle stem cells. In a further preferred form, the cardiac progenitor cells are cardiac progenitor cells. Preferably, the cells capable of forming the replacement tissue are autologous cells. In a particularly preferred form, the replacement tissue is a joint or part thereof.

In another preferred form, the scaffold for the joint comprises two opposing surfaces for growing bone and two faces each articulating with each opposing surface for growing cartilage, similar to a pseudarthrosis.

Preferably, the scaffold for the joint is placed under mechanical stress after it is implanted into a patient.

In a further broad form, the present invention relates to a scaffold for promoting in vivo growth of replacement tissue in a patient, wherein the scaffold is coated and/or

impregnated with cells capable of forming the replacement tissue and/or one or more growth factors for inducing differentiation of cells capable of forming the replacement tissue.

Preferably, the scaffold comprises ceramics (particularly soft ceramics), collagen, Hydroxyapatite, polymers such as polycaprolactone, plastics, fumaric acid derivatives, peptide-based biomaterials, blended polymers, polylactic acid, polylactite, polyethylene glycol, class A biomaterials, bioglass powders, highly hydrated soft gel-like materials, metallic substances such as stainless steel, copper, platinum, gold and/or tantalum.

In a particularly preferred form, the scaffold comprises a ceramic and collagen.

The scaffold may or may not have a surface charge or potential which attracts stem cells or osteoblasts or other types of cells.

In another preferred form, the scaffold is biodegradable.

In yet a further preferred form, the scaffold is non-biodegradable.

Even more preferably, the interior of the scaffold comprises a nanofibrous material that mimics the extracellular matrix of the tissue that is to be grown.

Preferably, the nanofibrous material of the scaffold is formed from self assembly, electrospinning, or phase separation.

In a particularly preferred form, the phase separation is thermally induced phase separation.

In another preferred form, the interior of the scaffold is solid.

Even more preferably, the scaffold is pre-shaped to fit a tissue defect using computer-

aided design.

In a particularly preferred form, selective laser melting is used to mould the final shape of the scaffold.

Preferably, where the replacement tissue is bone, selective laser melting is used to provide a pore size for the scaffold for promoting uncemented fixation of the scaffold and replacement bone, wherein the bone surrounding the replacement bone and scaffold grows into the scaffold and replacement bone.

In another preferred form, the scaffold is designed to replace joint cartilage in a patient.

Preferably, the joint cartilage is innoculated and/or coated with chondrocytes prior to implantation into a patient.

Even more preferably, the joint cartilage is selected from an intervertebral disc, a meniscal knee cartilage or hip-joint cartilage.

In a further preferred form, the scaffold is made from two or more different materials to support the growth of more than one type of tissue.

In another preferred form, the scaffold supports growth of a joint when placed in vivo.

Preferably, the scaffold is inoculated and/or coated with osteoblasts and/or chondrocytes prior to implantation into a patient.

Preferably, the joint is selected from a unicompartmental knee joint or a small joint of a hand. It would also be possible with a total knee replacement.

In another preferred form the scaffold is used to support growth of a segment of bone, wherein the segment of bone is grown in vivo and broken in vivo into two or more

portions, wherein the broken portions are placed under mechanical stress via normal anatomical movement and/or an artificial mechanical device, in order to induce the formation of one or more joints between the broken portions of bone.

In a further preferred form, micropieces of degradable matrices are suspended into a gel-like viscous fluids as an injectable scaffold mixture.

Preferably, the micropieces of degradable matrices injected into a bone defect to replace lost bone and/or cartilage.

Even more preferably, the micropieces of degradable matrices are injected into the defect using a minimally invasive surgical (MIS) technique.

In yet another broad form, the present invention relates to a bioactive coating for a biological implant, said coating comprising a three-dimensional nanoscaffold polymer that promotes union at an interface between a biological implant and a patient's own tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1: Schematic representation of an implanted joint scaffold in the latissimus dorsi muscle of a minipig.

FIGURE 2: Schematic representation of a joint being endocultivated in a human back inside the musculus latissimus dorsi.

FIGURE 3: X-ray of a Joint-Like Structure inserted into the musculus latissimus dorsi of a minipig during the endocultivation sequence of Figure 1. The arrow shows the region of articulating joint surfaces

FIGURE 4: Schematic diagram showing a bone formed in muscle mass being broken, and forming a joint between the bone ends if moved by muscle action or by other

mechanical means such as that depicted.

FIGURE 5: Schematic representation of placement of MIS knee incision.

FIGURE 6: Schematic representation showing location of axes of the femur and tibia.

FIGURES 7: Schematic example of placement of exemplary guides (or jigs) to perform replacement of knee components in the knee joint.

FIGURE 8: Schematic example of placement of exemplary guides (or jigs) to perform replacement of knee components in the knee joint.

FIGURE 9: Schematic example of placement of exemplary guides (or jigs) to perform replacement of knee components in the knee joint.

FIGURE 10: Schematic example of mechanisms of placement of replacement knee components into knee joint.

FIGURE 11: Schematic example of mechanisms of placement of replacement knee components into knee joint.

DETAILED DESCRIPTION OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the words "comprise," "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgement or any form of suggestion that the prior art forms part of the common general knowledge in Australia.

It has surprisingly been found by the present inventors that replacement tissue of any variety that is highly suitable for tissue grafting can be grown *in vivo* when a scaffold

that is coated and/or impregnated with cells capable of forming the replacement tissue and/or growth factors, is placed inside certain tissues in a patient, thus avoiding the need for harvesting tissue from the patient or a donor.

Accordingly the present invention includes a method for tissue regeneration for a patient, said method comprising:

a) providing a scaffold for the replacement tissue;

b) coating and/or inoculating the scaffold with cells and/or growth factors capable of forming the replacement tissue;

c) implanting the scaffold subcutaneously, intrafascially or into fat or muscle tissue in a host;

d) harvesting the scaffold, replacement tissue and a portion of the blood supply of the replacement tissue when sufficient tissue formation and angiogenesis of the replacement tissue has occurred;

e) transplanting or translocating the scaffold, replacement tissue and the blood supply of the replacement tissue to a site where the replacement tissue is required in the patient.

The term "patient" refers to patients of human or other mammal and includes any individual it is desired to examine or treat using the methods of the invention. However, it will be understood that "patient" does not imply that symptoms are present. Suitable mammals that fall within the scope of the invention include, but are not restricted to, primates, livestock animals (e.g., sheep, cows, horses, donkeys, pigs), laboratory test animals (e.g., rabbits, mice, rats, guinea pigs, hamsters), companion animals (e.g., cats, dogs) and captive wild animals (e.g., foxes, deer, dingoes).

Preferably, the cell type that is capable of forming the tissue that is to be grown in

vivo is a stem cell.

Even more preferably, the cell is an adult stem cell. An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ. Adult stem cells can self-renew, and can differentiate to yield the major specialised cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found.

A number of different stem cells populations have been isolated in adult tissue, any of which may be suitable for growing the replacement tissue according to the methods of the invention. These cells include, but are not limited to, satellite cells, which are skeletal muscle precursors (ie. myoblasts), as well as cardiac precursor cells (Laugwitz et al., Nature, 2005, 433, 10 Feb: 647-653), nasal stem cells, stromal cells (also known as mesenchymal cells, which can generate bone, cartilage, fat, and fibrous connective tissue (Pittenger et al., Science 2 Apr 1999, 284, 143-147), neuronal stem cells, chondrocytes, multipotent adult progenitor cells, peripheral blood stem cells, embryonic carcinoma cells isolated from teratocarcinomas, liver stem cells, pancreatic stem cells, corneal limbal stem cells, mammary stem cells, salivary gland stem cells and dermal hair follicle stem cells. The choice of stem cells will depend on the type of tissue that is sought to be grown in vivo.

The cells are often categorised by their source. "Autologous" cells come from the same body as that to which they will be reimplanted. "Allogenic" cells come from another body. "Xenogenic" cells come from another species.

Preferably, the methods of the invention employ autologous cells, in order to overcome the problems associated with rejection of foreign (ie. non-self) tissue in a patient.

A vast number of different growth factors exist in the human body. Growth factors

are proteins that bind to receptors on the cell surface, with the primary result of activating cellular proliferation and/or differentiation. Many growth factors are quite versatile, stimulating cellular division in numerous different cell types; while others are specific to a particular cell-type. These include, but are not limited to, PDGF, EGF, TGF-α, FGF, NGF, Erythropoietin, TGF-β (e.g. Bone Morphogenetic Proteins), IGF-I, IGF-II and proinsulin and IGF-II. The choice of growth factor used for the methods of the invention will, of course, depend on the tissue type that is being sought to be grown in vivo.

The term "translocation", as used herein, refers to movement of a scaffold, replacement tissue and its associated blood supply to a location that is in substantially close proximity to the site where the replacement tissue has grown in vivo, a process which thus negates a need to reconnect the blood supply of the replacement tissue into the site where the replacement tissue is required, due to the ability to move blood vessels a substantial distance without significant disruption of the vessels.

The term "transplantation", as used herein, refers to movement of a scaffold, replacement tissue and its associated blood supply to a location that is not in close proximity to the site where the replacement tissue has grown in vivo, a process which thus requires a need to reconnect the blood supply of the replacement tissue into the site where the replacement tissue is required, due to disruption (ie. disconnection) of the blood vessels of the replacement tissue.

The scaffolds for growing the replacement tissue may be implanted subcutaneously, below the periosteum, intrafascially or into adipose tissue or into muscle tissue.

The anatomical regions into which the scaffold may be implanted include subcutaneously, intrafascially, or into fat tissue or into a muscle.

Suitable muscles may be selected from muscles of the trunk selected from the group consisting of Trapezius, Latissimus dorsi, Pectoralis major, Pectoralis minor, Levator scapulae, Rhomboid minor, Rhomboid major, Serratus anterior, Deltoid, Supraspinatus, Infraspinatus, Teres minor, Teres major, Subscapularis, Splenius capitis, Splenius cervicis, Iliocostalis lumborum, Iliocostalis thoracis, Iliocostalis cervicis, Longissimus thoracis, Longissimus cervicis, Longissimus capitis, Spinalis thoracis, Spinalis cervicis: Spinalis capitis, Semispinalis thoracis, Semispinalis cervicis, Semispinalis capitus, Multifidus, Short rotators, Interspinalis, Intertransversi, Trapezius, Latissimus dorsi, Subclavius, Pectoralis major, Pectoralis minor, Levator scapulae, Rhomboid minor, Rhomboid major, Serratus anterior, Deltoid, Supraspinatus, Infraspinatus, Teres minor, Teres major, Subscapularis, Splenius capitis, Splenius cervicis, Iliocostalis lumborum, Iliocostalis thoracis, Iliocostalis cervicis, Longissimus thoracis, Longissimus cervicis, Longissimus capitis, Spinalis thoracis, Spinalis cervicis, Spinalis capitis, Semispinalis thoracis, Semispinalis cervicis, Semispinalis capitus, Multifidus, Long rotators, Short rotators and the Interspinalis, Intertransversi.

Alternatively, the muscle is a leg muscle selected from the group consisting of Tensor fascia lata, Gluteus maximus, Gluteus medius, Gluteus minimus, Piriformis, Superior gemellus, Obturator internus, Inferior gemellus, Quadratus femoris, Semitendinosus, 30 Adductor longus, Adductor brevis, Adductor magnus, Gracilis, Obturator externus, Sartorius, Rectus femoris, Vastus lateralis, Vastus intermedius, Vastus medialis, Articularis genus, Psoas major, Illiacus, Pectineus, Soleus, Plantaris, Popliteus, Flexor digitorum longus, Tibialis posterior, Flexor hallucis longus, Peroneus longus, Peroneus brevis, Tibialis anterior, Extensor hallucis longus, Extensor digitorum longus and Peroneus tertius.

In another form, the muscle is a head or neck muscle selected from the group consisting of Obliquus capitis inferior, Obliquus capitis superior, Rectus capitis posterior major, Rectus capitis posterior minor, Longus colli, Longus capitis, Rectus capitis anterior, Rectus capitis lateralis, Anterior scalene, Scalenus minimus (may be absent), Middle scalene, Posterior scalene, Sternocleidomastoid, Platysma, Sternohyoid, Omohyoid, Sternothyroid, Thyrohyoid, Stylohyoid, Digastric, Mylohyoid, Geniohyoid, Occipitalis (2 bellies), Frontalis (2 bellies), Orbicularis oculi, Corrugator supercilii, Levator labii superioris alaeque nasi, Levator labii superioris, Zygomaticus minor, Zygomaticus major, Risorius (may be absent), Levator angulioris, Buccinator, Depressor anguli oris, Depressor labii inferioris, Masseter, Medial pterygoid, Lateral pterygoid, Levator palpebrae superioris, Lateral rectus, Medial rectus, Superior rectus, Superior rectus, Inferior rectus, Superior oblique and the Inferior oblique.

In yet a further form, the muscle is an arm muscle selected from the group consisting of Coracobrachialis, Biceps brachii, Brachialis, Triceps brachii, Anconeus, Pronator teres, Flexor carpi radialis, Palmaris longus, Flexor carpi ulnaris, Flexor digitorum superficialis, Flexor digitorum profundus, Flexor pollicis longus, Pronator quadratus, Brachioradialis, Extensor carpi radialis longus, Extensor carpi radialis brevis, Extensor digitorum, Extensor digiti minimi, Extensor carpi ulnaris, Supinator, Abductor pollicis longus, Extensor pollicis brevis, Extensor pollicis longus, Extensor indicis, Abductor pollicis brevis, Flexor pollicis brevis, Opponens pollicis, Adductor pollicis, Palmaris brevis, Abductor digiti minimi, Flexor digiti minimi brevis, Opponens digiti minimi, Palmar interossei, Dorsal interossei, Lumbricals, Abductor hallucis, Flexor digitorum brevis, Abductor digiti minimi, Abductor ossis metatarsi quinti, Quadratus plantae, Lumbricals, Flexor hallucis brevis, Adductor hallucis,

Flexor digiti minimi brevis, Plantar interossei (3 muscles), Dorsal interossei (4 muscles), Extensor hallucis brevis and Extensor digitorum brevis.

Additionally, the vascular supply of the subcutaneous region, the intrafascial region, or the fat or the muscle containing the scaffold assists in angiogenesis of the newly grown tissue. Accordingly, the newly growing tissue is provided with a blood supply, and the blood vessels that grow into the graft tissue are harvested when sufficient angiogenesis and formation of replacement tissue has occurred, and connected to blood vessels at the site where the replacement tissue is required in the patient.

The term "sufficient tissue formation" as used herein refers to the formation within the scaffold of adequate amounts of graft tissue, as determined by extent tissue formation within or on the scaffold, as determined by any suitable methods in the art, such skeletal scintillography, as x-ray analysis and computerised tomography.

In order to shape the scaffold to fit the site where the replacement tissue is required, anatomical modelling studies can be employed, such as computed tomography or magnetic resonance imaging. Preferably, the anatomical modelling studies may further include use of computer–aided design. This is preferred because in order to achieve full functional loading, the nanoscaffolds or coated implants should be individually shaped to fit the desired host area perfectly.

Navigation of the placement of the expandable structure may be performed, for example, using BrainLAB. Probes and sensors may be placed on nearby anatomical landmarks. Navigation will guide the accurate placement of the scaffold into the tissue defect.

An imaging system is preferably employed to ensure correct placement of the replacement tissue that is grown in vivo. A number of suitable imaging systems exist in the field, and include, but are not limited to, radiolucent image intensifiers for

biplanar fluoroscopy, CT scanning as well as MRI.

The scaffolds of the invention are optimised at nanoscale, microscale and macroscale levels (the molecular, cellular and tissue levels respectively). Macroporous features of the scaffold define the initial void space that is available for cell migration and ingrowth of new blood vessels. Microstructural features define the surface texture that cells encounter, which can have important biologic effects on cell functions. Features at all levels influence fluid flow and diffusion of oxygen and other nutrients through the scaffold.

An example of such a feature which can be used is rapid removal of fluid from hyperhydrated collagen gel (or other) constructs using plastic compression fabrication. (Brown RA et al., Adv. Funct. Mater., 2005, 15, 1762-1770) which allows rapid engineering of desired tissues with controllable nano and micro scale biomimetic structures with high cell viability.

The extracellular matrices (ECMs) of hard tissue are composed on organic and inorganic phases, the inorganic phase consisting mainly of hydroxyapatite and the organic phase consisting of mainly type 1 collagen. It is well known that tissue integrity and cell survival depend on the pore size and diffusion distances inside the extracellular matrix (ECM). Furthermore, the fibrillar structure of collagen ECM is important for cell attachment, proliferation and differentiated function in tissue culture. As the scaffolds of the invention mimic the natural structure of the tissue that is to be grown in vivo using the scaffold, they lead to tissue formation more closely resembling native tissues.

Various methods are known that can achieve nanofibrous materials for the scaffolds, that permit them to act as artificial extra-cellular matrices. These techniques include

self assembly, electrospinning and phase separation.

Preferably, the technique used is thermally induced phase separation. Controlled phase separation is a method has been used for years in the preparation of porous polymer membranes. Thermally induced phase separation is a newer technique for the preparation of porous membranes. The fabrication of foam scaffolds occurs in 5 basic steps: 1. Polymer dissolution 2. Phase separation and gelation 3. Solvent extraction from the gel with water 4. Freezing 5. Freeze drying under vacuum.

Scaffolds made using phase separation matrices contain 3 size scales: macropores (ie. macropore distribution), interfiber distance and fiber diameter. Nanofibrous scaffolds formed with phase separation provide a great deal of control and diversity in the scaffolds. Everything from fiber diameter to an interconnected pore structure can be controlled in order to tailor the scaffold to any application. The 3D pore network can also be built into the scaffold construct. Such fine control over the system, permits control over batch-to-batch consistency.

The scaffolds of the invention may be biodegradable or non-biodegradable.

Examples of suitable materials for the scaffolds include, but are not limited to, ceramics (particularly soft ceramics), collagen, hydroxyapatite, polymers such as polycaprolactone, plastics, fumaric acid derivatives, peptide-based biomaterials, blended polymers, polylactic acid, polylactite, polyethylene glycol, class A biomaterials, bioglass powders, highly hydrated soft gel-like materials, metallic substances such as stainless steel, copper, platinum, gold and/or tantalum.

In a particularly preferred form, the scaffold comprises a soft ceramic and collagen.

In a further form, the scaffold materials may be used as a three-dimensional nanoscaffold polymer coating, which can be used to coat scaffolds and other

implants (eg. artificial joint implants or dental implants) and act as a bioactive surface that enhances the interface between the implant and the patient's own tissue by improving the union between the surface of the biological implant and the patient's own tissue.

The mechanical properties of a scaffold must be matched to the graft environment and demands. The scaffolds are fashioned from porous materials that can be tailored at the nanoscale and processed to control porosity. An example of such scaffolds are a 4 – 12 micron polycaprolactone nanoscaffold and nanostructured porous polymer scaffold, which have been developed to support the in vivo growth of bone tissue.

In a particularly preferred form, the scaffold is designed to replace joint cartilage in a patient, and may be innoculated and/or coated with chondrocytes prior to implantation into a patient. Alternatively or additionally, the scaffold may be innoculated and/or coated with chondrocyte growth factors prior to implantation into a patient.

Particularly preferred replacement joint cartilage is an intervertebral disc, a meniscal knee cartilage or hip-joint cartilage.

In a particularly preferred form, the scaffolds of the inventions are made from different materials, and referred to herein as "hybrid" scaffolds, and can be used to support the growth of more than one type of tissue, such as both bone and cartilage growth.

Thus, in a particularly preferred form, the scaffold of the invention is designed to grown a replacement joint in vivo in a patient. Such a scaffold may be pre-loaded with osteoblasts and chondrocytes prior to implantation into a patient, and/or be impregnated/innoculated or coted with suitable growth factor/s that induce differentiation of precursor cells into osteoblasts and chondrocytes. The tissue grown

according to this embodiment will thus have a sandwich-structure of a cartilage layer surrounded by two layers of bone.

It is preferable that mechanical stress is applied to the joint scaffold after it is implanted into the patient in order to encourage formation of a functional false joint. Mechanical stress may be applied by normal body movement or a machine. Further mechanical loading through secondary surgical intervention may be used to form a joint space.

In a further preferred form, a segment of bone grown in vivo may be broken or cut during or after first endocultivation sequence, and the broken segments may then be placed under mechanical stress via normal anatomical movement and/or an artificial mechanical device, in order to induce the formation of a joint between the broken segments of bone, ie. pseudoarthrosis (See Figure 4). A pseudarthrosis can lead into a synarthrosis like joint for subsequent replacement of a synarthrosis joint. Alternatively, the pseudarthrosis or synarthrosis can be later divided and prepared to form a joint space and two articulating joint surfaces under mechanical loading. It is optional to add cartilage forming techniques like ACI to the endocultivated joint.

In a preferred form a suitable form of cartilage or stem cells are injected under a membrane which is sutured or held to the sides of the bony surface, thereby creating a layer of chondrocyte cells on the biomimetic scaffold suitable for chondrocytes and held in place by the biodegradable membrane until in growth occurs.

Preferably, Selective Laser Melting (SLM) rapid prototyping processes are used to manufacture the shaped scaffolds of the invention from the starting material that is preferably manufactured using phase separation. These involve a very precise nano laser beam of only 0.03 mm (30μ) diameter which is able to build metal structures of

only 0.05 mm part size in any complexity. The implants can be either made solid or in a micromesh, sponge-like structure to allow ingrowth of tissue. Ultra light mesh structures are some of the most complex and dimensionally demanding geometries ever generated in metals, with over 450 holes and channels per cm^3. Giving an implant a micromesh structure leads to 90% reduction in weight while still retaining enormous strength and stiffness with the material. In the same laser melting process a combination with biomimetic nanoscaffold surfaces can be achieved (ie. coating of the scaffold with the biometric three-dimensional polymer of the invention).

Selective Laser Melting may be used to build interconnecting 3 dimensional micromesh structures from various ceramics including hydroxyapatite, which is biodegradable. The method of forming hydroxyapatite nanoscaffold constructs using the selective laser melting technology is to use a very low wattage laser thus enabling the ceramic to remain intact and retaining its designed porous structure during the manufacturing process and / or mixing metal (for example titanium) powder with the ceramic powder in the hopper so that during the manufacturing process the heat generated is absorbed by the metal particles thus leaving the ceramic or softer component undisturbed. The advantage of using precise CAD design is that the scaffold can be customised to fit perfectly into the tissue defect in the patient. Thus there is no need for cementation or invasive fixation techniques.

In a particularly preferred form the ceramic scaffold block ranges from a hard bearing surface at one end to a porous ingrowth surface at the opposite end, whereby the porous surface is fitted onto suitable bone stock for bony ingrowth and fixation and the smooth hard polished surface at the opposite end of the scaffold block is used as a bearing or articulation or outer surface.

Where the tissue is bone, it is preferable that the implant is transplanted to the site where the replacement tissue is required and retained there via uncemented fixation. Preferably, selective laser melting is used to provide a pore size for the scaffold for promoting uncemented fixation of the scaffold and replacement bone, wherein the bone surrounding the replacement bone and scaffold grows into the scaffold and replacement bone. In this regard, the scaffold and replacement bone are incorporated into the structure of the whole bone. Furthermore, the pore size created by selective laser melting allows the replacement bone to remodel and respond to stress in a manner substantially similar to that of normal bone.

In a further form, micropieces of degradable matrices will be designed as well. These micropieces will be suspended into gel-like viscous fluids which will result in injectable scaffold mixtures. Such injectable scaffold mixtures are preferably used to fill a bone defect in order to replace lost bone and/or cartilage within the defect. Preferably, the micropieces of degradable matrices will be injected using minimally invasive surgical (MIS) methods.

In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

EXAMPLES

EXAMPLE 1A

Biocompatibility testing of 3 dimensional matrices in vitro

A scaffold for in vivo tissue growth comprising collagen and different compositions of polymeric mixtures are manufactured. As the scaffold requires biocompatibility in human cell cultures, the proliferation and cell survival of human keratinocytes,

fibroblasts, osteoblasts, chondrocytes and mesenchymal stem cells on the scaffold are validated by light and electron microscopy. The most biocompatible collagen/polymeric mixture is chosen for in vivo use.

EXAMPLE 1B

Biocompatibility and mechanical testing of matrices in vivo

The selected scaffold was implanted into a pouch of the Musculus latissimus dorsi of a minipig (Figure 1) to grow bone and cartilage and also allow for ingrowth of vessels from the thoracodorsal artery. Two techniques are applied. The scaffold is either be preloaded with osteoblasts/ chondrocytes prior to implantation or receives a suitable growth factor (as per the joint shown in Figure 3), to induce differentiation of precursor cells into osteoblasts or chondrocytes. The induced tissue has a sandwich tructure of a cartilage layer surrounded by two layers of bone. The quality and growth sequences of the tissue are validated histomorphometrically. Core biopsies of the mineralized tissue are tested for elasticity, compression, shear and tensile stability in mechanical testing devices.

EXAMPLE 1C

Growth and transplantation of a vascularised joint in vivo.

The optimal three-dimensional scaffold has similar mechanical integrity as natural bone and cartilage. The scaffold was individually shaped to replace a suitable joint in a minipig. The scaffold has two bases of bone and two articulating cartilage surfaces in the middle. This joint-like structure (JLS) was prefabricated (endocultivated) inside the M latissimus dorsi of minipigs to achieve a vessel pedicle formed out of the thoracodorsal artery. Mechanical manipulation of the joint in vivo may be

achieved through anatomical movement. Alternatively, a segment of bone grown in vivo (eg. in a muscle mass) may be broken, and mechanical manipulation of the segments of broken bone via normal anatomical movement or using a mechanical device, such as that shown in Figure 4, will induce the formation of a joint between the broken pieces of bone (ie. pseudoarthrosis). The pseudarthrosis is similar to a synarthosis like joint and can be used to replace a synarthrosis. Alternatively, the pseudarthrosis or synarthrosis can be later divided and prepared to form a joint space and two articulating joint surfaces (diarthrosis) under mechanical loading. It is optional to add cartilage forming techniques like ACI, where mesenchymal stem cells or chondrocyte cells harvested to yield maximum cartilage are loaded onto the scaffold either under a membrane (eg Chondrogide) or sprayed on under sufficient pressure to enable sticking to the endocultivated joint surfaces.

After the endocultivation or prefabrication period, the JLS is transplanted to replace the hip joint of the minipig. The adjacent muscle tissue and the vessel pedicle will be harvested simultaneously, allowing for transplant blood perfusion after re-anastomosis of the vessels in the recipient region. The surrounding muscle tissue will also serve as a pseudo joint capsule. The JLS will receive full functional loading after transplantion. Alternatively a joint capsule and bone periosteum can be formed from fascia or engineered periosteum.

An example for such a procedure in humans can be summarized as follows:

Step 1 will be a virtual Computer Model of an individually shaped joint replacement for the patient in order to manufacture an analogous growth-guiding scaffold. This model will be designed based on CT and / or MRI data of the patients joint or area of

defect and will fit exactly into the diseased area, which will later be removed during surgery.

Step 2 is the implantation of the customized scaffold together with growth factors/cytokines and pluripotent adult stem cells in the Latissimus dorsi muscle of the patient. Thus, the patient is their own bioreactor and regenerates their own tissue to grow the JLS. Some loading will take place during the incubation stage with the use of mechanical arms.Surgical interventions during endocultivation period will form a pseudarthrosis/synarthrosis and/or articulating joint surfaces (diarthrosis).

Step 3 is the transplantation of the JLS with adjacent vessel pedicle into the recipient region to replace the original joint. Immediate functional loading shall be achieved as already demonstrated in our basic mandible studies. Functional loading should lead to remodelling and total integration/incorporation of the JLS into the patient.

In addition, with this technique it will be possible to endocultivate all kinds of tissue (and complex organs) which allow for ingrowth of a blood supply. Scaffolds or matrices can be loaded with liver cells or stem cells during implantation or prior to implantation into the living bioreactor, where the endocultivation sequence inside the patient is performed. During the endocultivation sequence the vessels will grow into the cell loaded scaffold and give a blood supply to the growing tissue.Thus, this technique will allow for growth of large tissue quantities and for subsequent transplantation or translocation after cultivation period. For example, when liver tissue has grown at the endocultivation site it can be subsequently transplanted into the recipient region (portal vein blood circuit). At the recipient site the tissue will start function of the desired organ.

EXAMPLE 2

In vivo Growth of Replacement Knee Joint in a Human

Surgical Instructions

Design Rationale:

This in vivo tissue engineering method employs a minimally invasive surgery (MIS) technique for knee joint replacement (partial/uni-, total knee replacement and patellofemoral arthroplasty). The advantages of MIS surgery are well established, and include size and approach of the incision. Other advantages of MIS surgery for knee replacement applications are discussed below.

A "docking system" allows precise and stable placement of the implant. The advantages of this surgical approach are:

1. 1. The benefits of MIS Knee Surgery (see below).
2. 2. The Benefits of the Docking System used (see below).
3. In-vivo tissue engineering uses the patient's own tissue and so avoids the problems of bone loss, rejection and complications of mechanical implant wear.

This implant is a ceramic-ceramic/ or cartilage in-vivo bone sandwich, although a metal with PE insert option is available. It holds and integrates at the bony interface, rather than at the implant/bony interface.

Navigation is optional.

Indications:

This surgical approach is indicated for knee replacement in the following conditions, provided there is sufficient femoral and tibial bone stock:

-Osteoarthritis

-Rheumatoid arthritis

-Osteonecrosis

Contraindications:

This surgical methodology should not be used where:

-MIS techniques are contra-indicated

-The patient is obese, making the palpation of anatomical landmarks difficult. In revision procedures until sufficient experience is achieved.

-Other standard contraindications to knee replacement still apply.

Advantages of MIS Knee Surgery:

-a smaller (6cm) more cosmetic scar hidden medially.

-the incision is anatomical and placed far from the tibial tubercle with the reduced chance of wound healing problems.

-quads sparing,(patella is NOT dislocated)

-avoids intra-operative knee dislocation/subluxation

-more accurate patella balancing

-reduced blood loss -less risk of infection

-possible reduced incidence of clots (DVTs) -reduced hospital stay and costs

-it may be done as day or outpatient surgery

Advanatges of the unified "docking" guidance/shock absorption system:

-precise and unified placement of the components.

-uses an instrument or frame (the EPISTART, as described as the frame for application to a knee joint during knee replacement surgery in WO04/100839, the

contents of which is incorporated herein in its entirety) which identifies the epicondylar axis (essential axis) of the femur/knee.

-re-defines our view of the knee in terms of its ligament attachments rather than from the slope of the joint line (avoids risky IM rod for femur).

–immediate mechanical fixation (early fixation). No cement required.

-early bony incorporation and fixation (earlier long- term fixation)

-'impact dispersal' to reduce impact/shear forces across the articular surfaces and so reduces long-term wear and premature aseptic loosening of the components.

-achieves soft tissue tension balancing of both the flexion, extension and patellar spaces/gaps. -Navigation Systems are optional extra (surgeon's choice)

Components: -The Instrument System. Universal (requring modification of current implants). Customised operation.

-The implant (ie. scaffold) -the EPISTART instrument

Preoperative Planning:

The standard pre-operative planning for a knee replacement still applies.

Patient Positioning:

This system uses the medial approach. The patient is positioned in the supine position with the knee flexed. Tourniquet use is optional.

Operative Technique:

STEP ONE: in vivo placement of manufactured scaffold

Determine the size of the knee components required (the range of sizes is reduced).

A 5-cm incision is made over the adductor muscle mass and place the prepared scaffold within the muscle mass. The scaffold is left for six weeks and bony growth

is reviewed with CT imaging. Alternatively, the scaffold can be incubated in another part of the body such as by the ilium bone of the pelvis.

STEP TWO: retrieval of the scaffold with grown replacement tissue

Return at six weeks and use the same incision to retrieve the scaffold and its attached replacement tissue.

STEP THREE: the MIS Knee incision

Place a 6cm C(drop) - incision, medial parapatellar to the tibial edge. Incise the capsule 2cm cranial and 2-3 cm caudally along the anterior border of the medial collateral ligament (Figure 5). It is so placed far away from the tibial tubercle. The extensor mechanism of the knee is therefore not disturbed.

Note: Define and locate the epicondylar axis of the lower femur (the EPISTART instrument is provided) and the vertical (perpendicular) axis of the tibia (Figure 6). (Surgeon may also elect to use the posterior condylar axis or tibial axis).

STEP FOUR: application of the loop frame

Apply the loop frame of the EPISTART instrument about the epicondylar axis (Figure 7). The loop frame is parallel to the OR Table and the floor. The knee is extended. Note that the femoral and tibial axes are parallel and perpendicular to the vertical (perpendicular) axis of the tibia. The distance between the axes depends upon the radius of curvature of the femoral component, the minimal thickness of the PE insert and the thickness of the tibial component.

STEP FIVE: application of the jigs

This Knee jig is applied to the side of the loop frame (Figure 8). The three soft tissue guides are inserted through the jig.

STEP SIX: building the docking platform

The lower femur is cored to build the femoral docking platform (3 monorails) a further guide wire is placed across the lower femur (Figure 8). There is now a guide for the osteotomy of the lower femur.

STEP SEVEN: preparation of the femur and tibia

-Femur: The osteotomy of the lower femur is performed (Figure 9).

-Tibia: The upper tibia is prepared with the jig. It is correlated with the optimal tension of the PCL. It is applied to build the tibial docking platform (two monorails). Then the osteotomy is performed (Figure 10).

This tibial docking platform can be performed in full extension or flexion

STEP EIGHT: placement of the scaffold and replacement tissue

Trial components are placed and the knee tested for range of movement (ROM) and stability.

The final knee replacement components are placed in this order: femoral and then tibial. These femoral and tibial components are docked (slid in) from the medial side along the monorails (Figure 10).

STEP NINE: patellar placement

Check the knee for ROM and stability. If patellar is to be replaced, prepare with 3-jig and dock final patellar component (two rails) (Figure 11).

Persons skilled in the art will appreciate that numerous variations and modifications will become apparent. All such variations and modifications which become apparent to persons skilled in the art, should be considered to fall within the spirit and scope that the invention broadly appearing before described.

CLAIMS

1. A method for tissue regeneration for a patient, said method comprising:

a) providing a scaffold for the replacement tissue;

b) coating and/or inoculating the scaffold with cells and/or growth/differentiating/morphogenetic factors(cytokines) capable of forming or inducing formation of the replacement tissue;

c) implanting the scaffold subcutaneously on contact to a fascia, below the pereosteum, or into fat or muscle tissue in a host;

d) harvesting the scaffold, replacement tissue and a portion of the blood supply of the replacement tissue when sufficient tissue formation and angiogenesis of the replacement tissue has occurred;

e) transplanting or translocation of the scaffold, replacement tissue and the blood supply of the replacement tissue to a site where the replacement tissue is required in the patient; and

f) connecting at least part of a blood supply of the replacement tissue to a blood vessel at the site where the replacement tissue is required.

2. A method for tissue regeneration for a patient in accordance with claim 1, characterised in that the scaffold is moulded to a given size and shape, said size and shape being substantially the same as the site where the replacement tissue is required.

3. A method for tissue regeneration for a patient in accordance with claim 1 or 2, characterised in that the cells capable of forming the replacement tissue are selected

from osteoblasts, osteoblast precursor cells, skeletal muscle precursors (myoblasts), periosteal cells and periosteal stem cells, cardiac and endothelial progenitor cells, nasal stem cells, stromal cells, neuronal stem cells, chondrocytes, multipotent adult progenitor cells, peripheral blood stem cells, embryonic carcinoma cells isolated from teratocarcinomas, liver stem cells, pancreatic stem cells, corneal limbal stem cells, mammary stem cells, salivary gland stem cells and dermal hair follicle stem cells.

4. A method for tissue regeneration for a patient in accordance with claim 3, characterised in that the progenitor cells are cardiac progenitor cells.

5. A method for tissue regeneration for a patient in accordance with any one of the preceding claims, characterised in that the cells capable of forming the replacement tissue are autologous cells.

6. A method for tissue regeneration for a patient in accordance with any one of the preceding claims, characterised in that the replacement tissue is a joint or part thereof.

7. A method for tissue regeneration for a patient in accordance with claim 6, characterised in that the scaffold for the joint comprises two opposing surfaces for growing bone and two faces each articulating with each opposing surface for growing cartilage, similar to a pseudarthrosis.

8. A method for tissue regeneration for a patient in accordance with claim 7, characterised in that the scaffold for the joint is placed under mechanical stress after it

is implanted into a patient.

9. A method for tissue regeneration for a patient in accordance with any one of the preceding claims, characterised in that that the scaffold is pre-shaped to fit a tissue defect using computer-aided design.

10. A method for tissue regeneration for a patient in accordance with any one of the preceding claims, characterised in that a surface charge or potential is induced in the scaffold which attracts stem cells or osteoblasts or other types of cells.

11. A method for tissue regeneration for a patient in accordance with any one of the preceding claims, characterised in that the scaffold is nanofibrous material formed from self assembly, electrospinning, or phase separation.

12. A method for tissue regeneration for a patient in accordance with claim 11, characterised in that the phase separation is thermally induced phase separation.

13. A method for tissue regeneration for a patient in accordance with any one of the preceding claims, characterised in that selective laser melting is used to mould the final shape of the scaffold.

14. A method for tissue regeneration for a patient in accordance with claim 13, characterised in that where the replacement tissue is bone, the selective laser melting is used to provide a pore size for the scaffold for promoting uncemented fixation of the scaffold and replacement bone, wherein the bone surrounding the replacement bone and scaffold grows into the scaffold and replacement bone.

15. A scaffold for promoting in vivo regeneration of replacement tissue in a patient, wherein the scaffold is coated and/or impregnated with cells capable of forming the replacement tissue and/or one or more growth factors for inducing differentiation of cells capable of forming the replacement tissue.

16. A scaffold in accordance with claim 15, characterised in that the scaffold comprises ceramics (particularly soft ceramics), collagen, Hydroxyapatite, polymers such as polycaprolactone, plastics, fumaric acid derivatives, peptide-based biomaterials, blended polymers, polylactic acid, polylactite, polyethylene glycol, class A biomaterials, bioglass powders, highly hydrated soft gel-like materials, metallic substances such as stainless steel, copper, platinum, gold and/or tantalum.

17. A scaffold in accordance with claim 15, characterised in that the scaffold comprises a ceramic and collagen.

18. A scaffold in accordance with any one of claims 15 to 17, characterised in that the scaffold has a surface charge or potential which attracts stem cells or osteoblasts or other types of cells.

19. A scaffold in accordance with any one of claims 15 to 18, characterised in that the scaffold is biodegradable.

20. A scaffold in accordance with any one of claims 15 to 19, characterised in that the interior of the scaffold comprises a nanofibrous material that mimics the extracellular matrix of the tissue that is to be grown.

21. A scaffold in accordance with claim 20, characterised in that the nanofibrous material of the scaffold is formed from self assembly, electrospinning, or phase separation.

22. A scaffold in accordance with claim 21, characterised in that the phase separation is thermally induced phase separation.

23. A scaffold in accordance with claim 15, characterised in that the interior of the scaffold is solid.

24. A scaffold in accordance with any one of claims 15 to 23, characterised in that the scaffold is pre-shaped to fit a tissue defect using computer-aided design.

25. A scaffold in accordance with any one of claims 15 to 24, characterised in that selective laser melting is used to mould the final shape of the scaffold.

26. A scaffold in accordance with claim 25, characterised in that where the replacement tissue is bone, selective laser melting is used to provide a pore size for the scaffold for promoting uncemented fixation of the scaffold and replacement bone, wherein the bone surrounding the replacement bone and scaffold grows into the scaffold and replacement bone.

27. A scaffold in accordance with any one of claims 15 to 26, characterised in that the scaffold is made from two or more different materials to support the growth of

more than one type of tissue.

28. A scaffold in accordance with any one of claims 15 to 27, characterised in that the scaffold is designed to replace joint cartilage in a patient.

29. A scaffold in accordance with claim 28, characterised in that the joint cartilage is innoculated and/or coated with chondrocytes prior to implantation into a patient.

30. A scaffold in accordance with claim 29, characterised in that the joint cartilage is selected from an intervertebral disc, a meniscal knee cartilage or hip-joint cartilage.

31. A scaffold in accordance with claim 30, characterised in that the scaffold supports growth of a joint when placed in vivo.

32. A scaffold in accordance with claim 31, characterised in that the scaffold is inoculated and/or coated with osteoblasts and/or chondrocytes prior to implantation into a patient.

33. A scaffold in accordance with claim 15 characterised in that the joint is a unicompartmental knee joint or a small joint of a hand.

34. A scaffold in accordance with claim 15, characterised in that the scaffold is used to support growth of a segment of bone, wherein the segment of bone is grown in vivo and broken in vivo into two or more portions, wherein the broken portions are placed under mechanical stress via normal anatomical movement and/or an artificial mechanical device, in order to induce the formation of one or more joints between the

broken portions of bone.

35. A scaffold in accordance with claim 34, characterised in micropieces of degradable matrices are suspended into a gel-like viscous fluids as an injectable scaffold mixture.

36. A scaffold in accordance with claim 35, characterised in that the micropieces of degradable matrices injected into a bone defect to replace lost bone and/or cartilage.

37. A scaffold in accordance with claim 36, characterised in that the micropieces of degradable matrices are injected into the defect using a minimally invasive surgical (MIS) technique.

38. A bioactive coating for a biological implant, said coating comprising a three-dimensional nanoscaffold polymer that promotes union at an interface between a biological implant and a patient's own tissue.

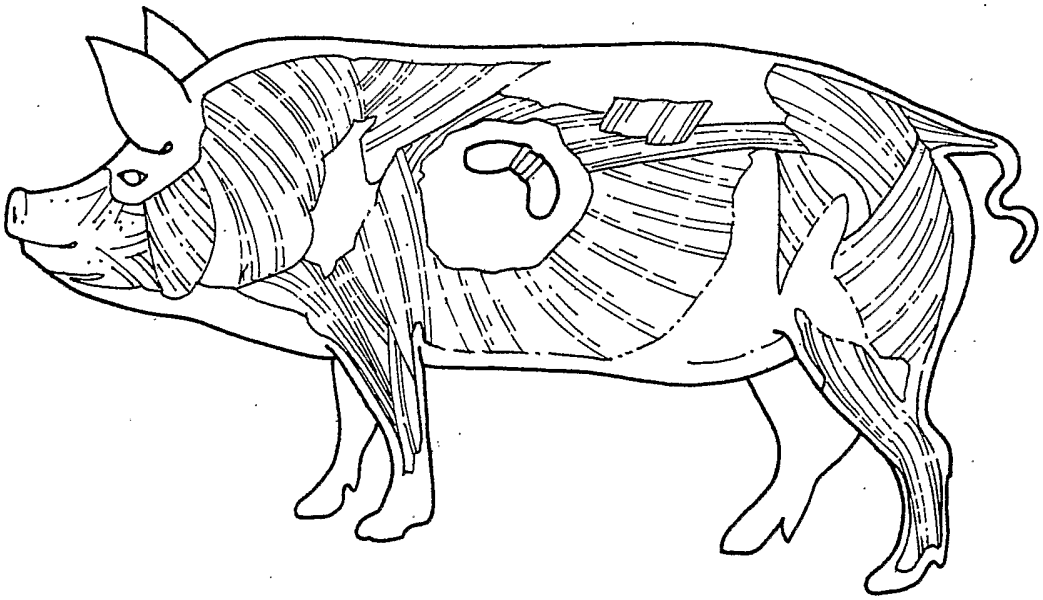

FIG. 1

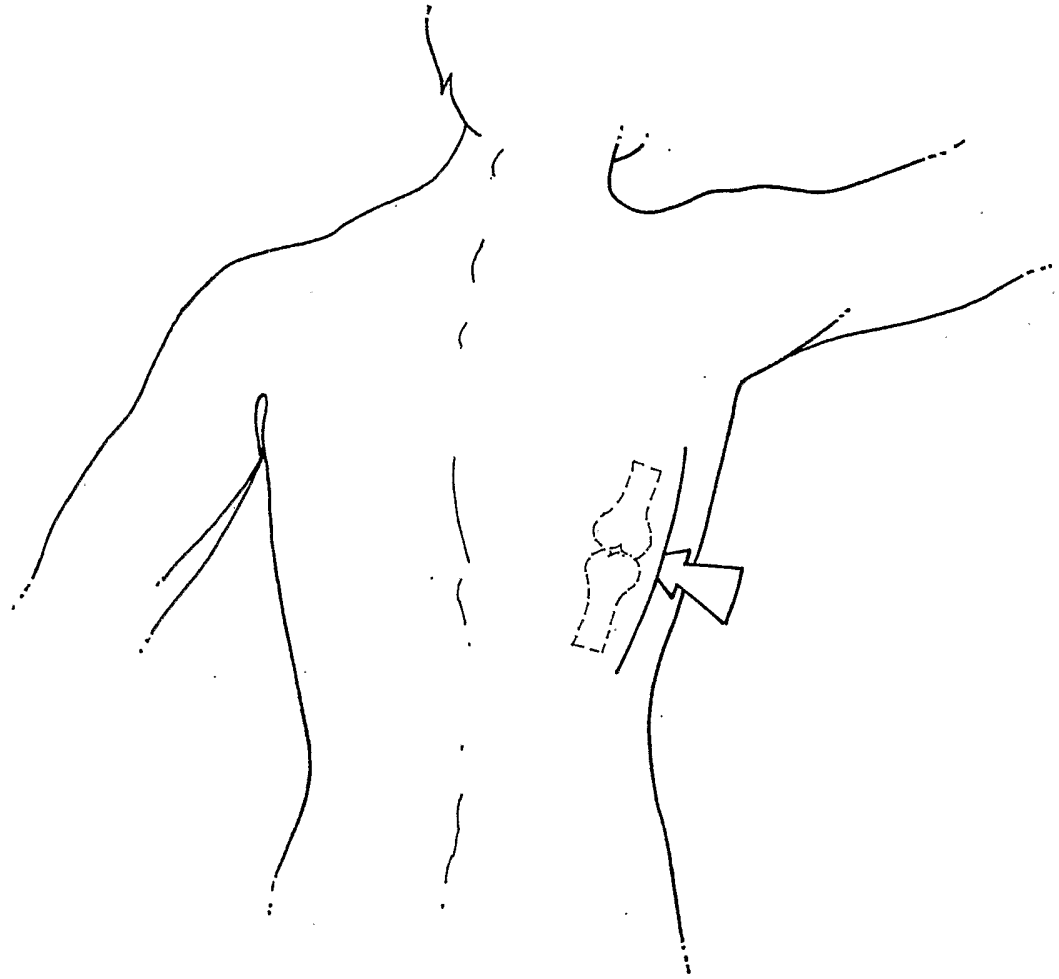

FIG. 2

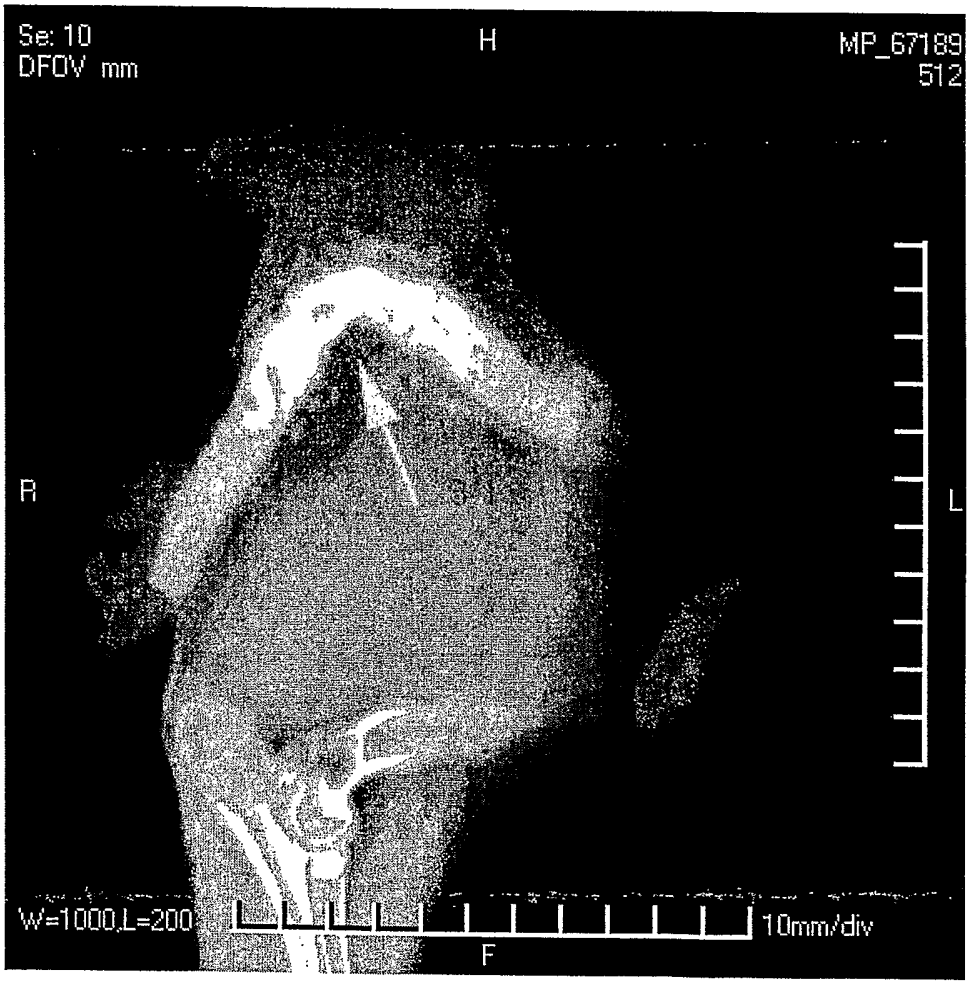

FIG 3.

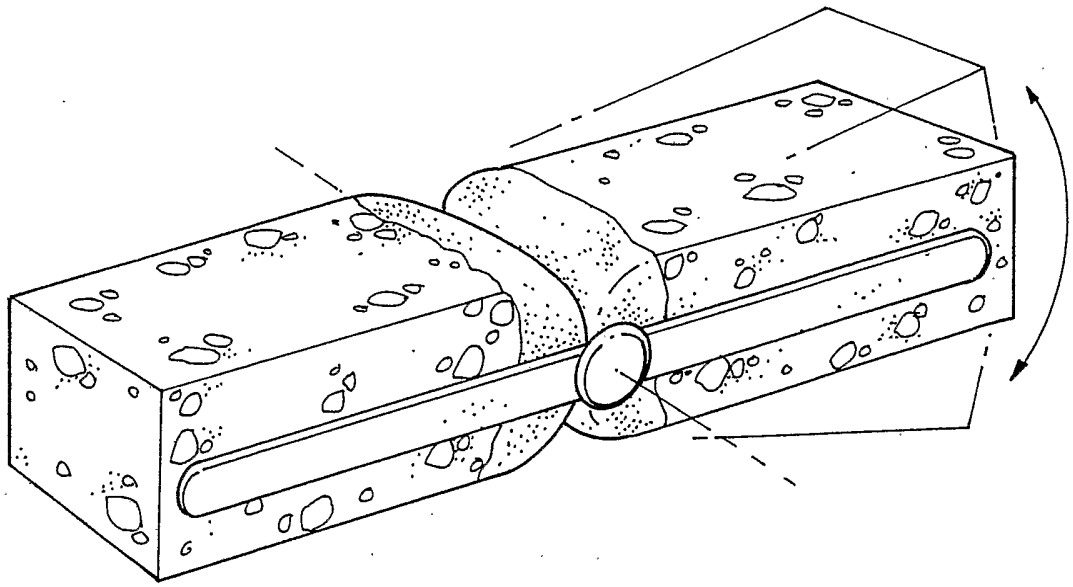

FIG. 4

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FIGURE 5

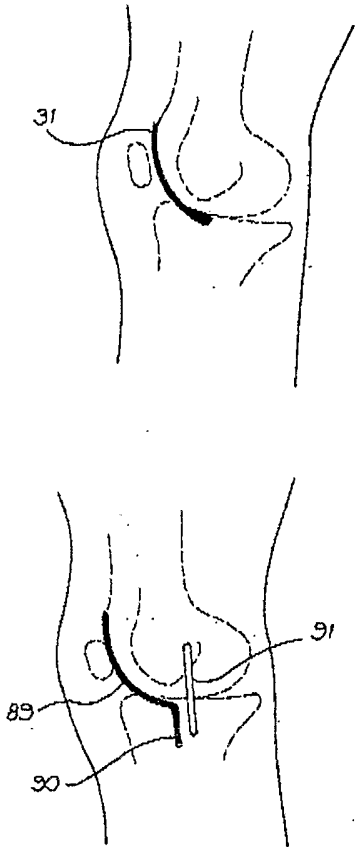

FIGURE 6

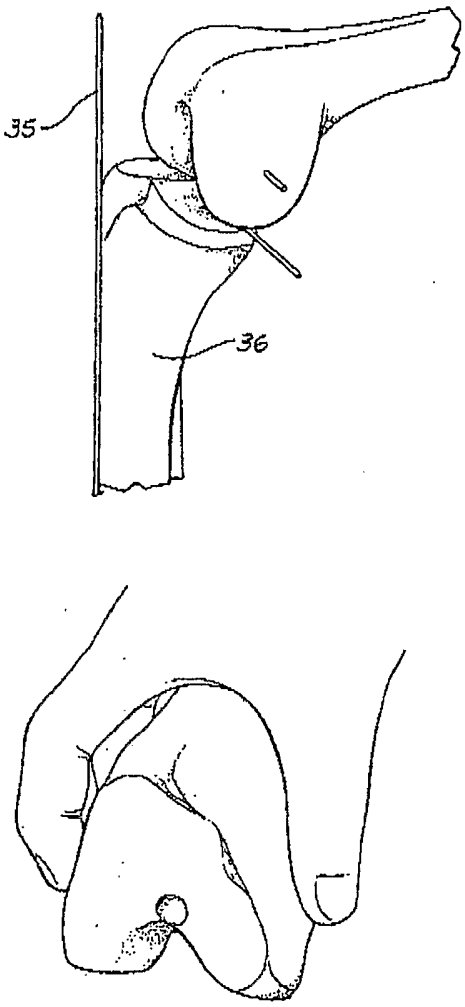

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FIGURE 7

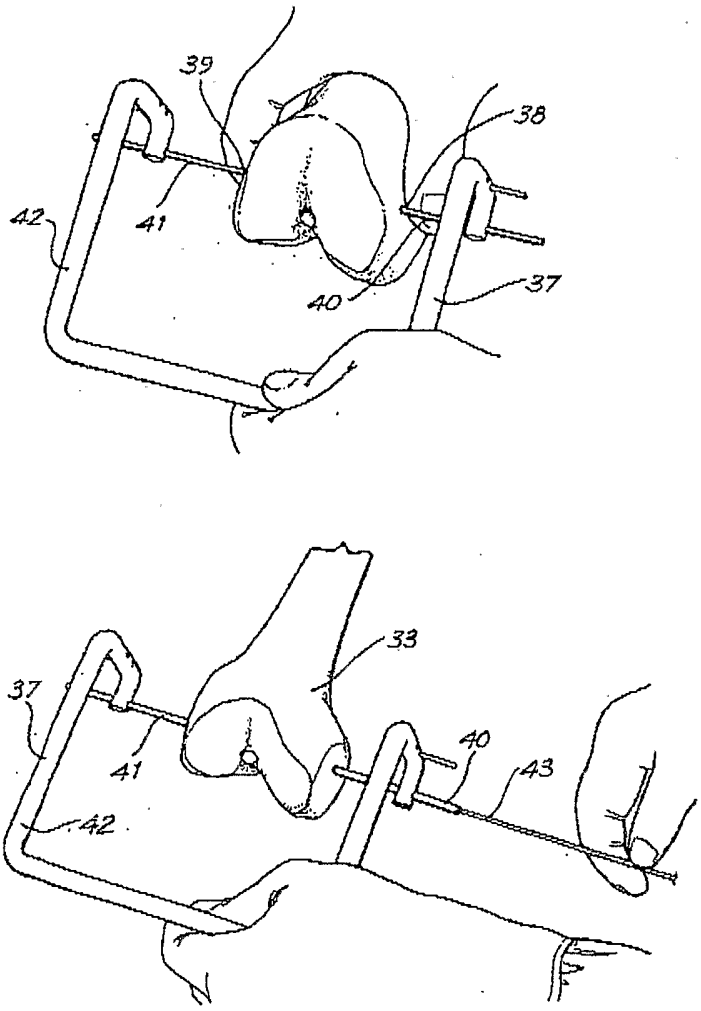

FIGURE 8

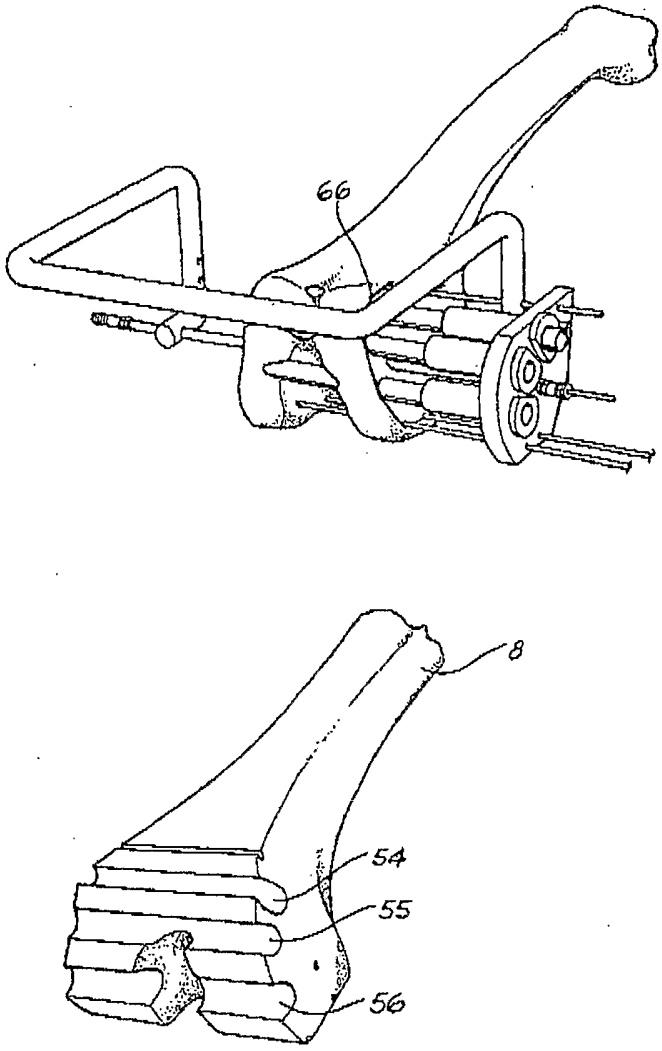

FIGURE 9

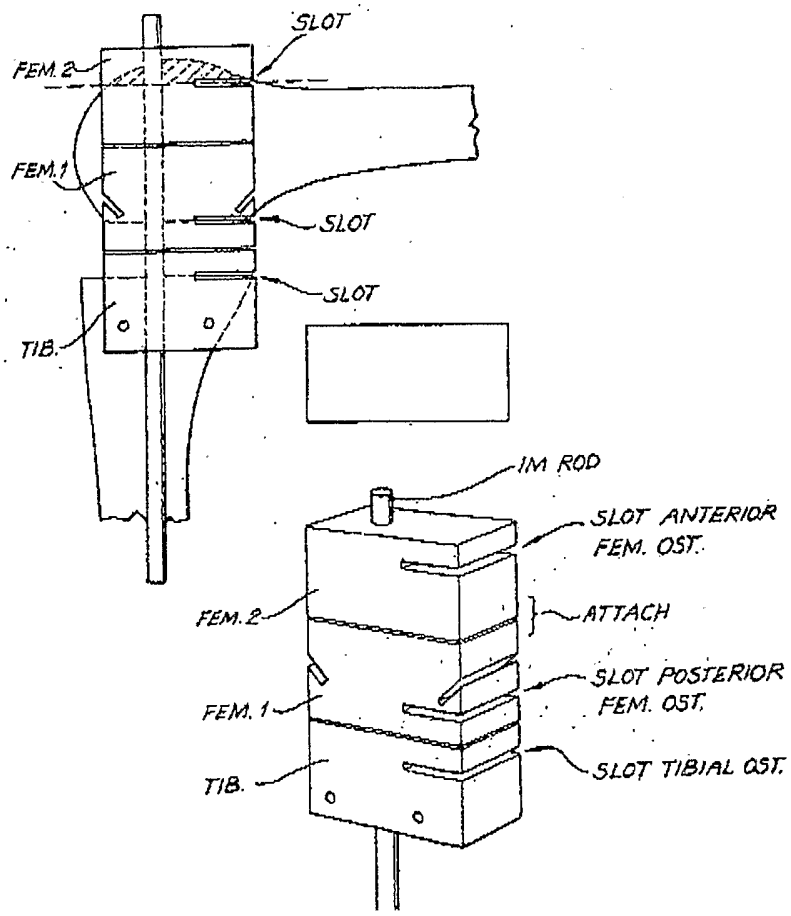
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FIGURE 10

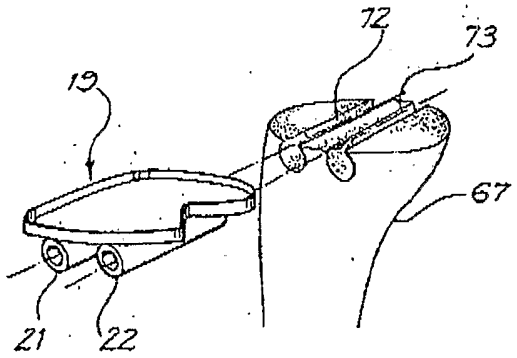

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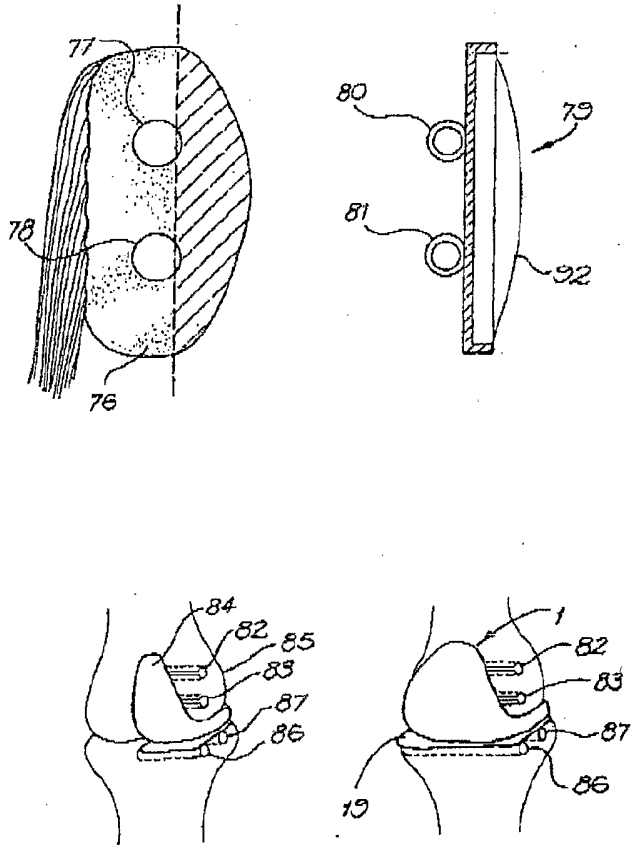
FIGURE 11



INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2006/000837

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L27/38 C12N5/06

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WARNKE ET AL: "Man as living bioreactor: Fate of an exogenously prepared customized tissue-engineered mandible" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 27, no. 17, June 2006 (2006-06), pages 3163-3167, XP005324791 ISSN: 0142-9612 the whole document	1-38
P,X	WARNKE ET AL: "The mechanical integrity of in vivo engineered heterotopic bone" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 27, no. 7, March 2006 (2006-03), pages 1081-1087, XP005152648 ISSN: 0142-9612 the whole document	1-38

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 July 2006	08/08/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fayos, C

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	HOLT G E ET AL: "Evolution of an in vivo bioreactor" JOURNAL OF ORTHOPAEDIC RESEARCH, THE JOURNAL OF BONE AND JOINT SURGERY, INC.,, US, vol. 23, no. 4, July 2005 (2005-07), pages 916-923, XP004977057 ISSN: 0736-0266 abstract page 921, right-hand column, last paragraph - page 922, left-hand column, paragraphs 1,2	1-38
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Y	US 5 711 957 A (PATAT ET AL) 27 January 1998 (1998-01-27)	1-14
X	claims 1-24	15-37

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 067 963 A (KHOURI ET AL) 26 November 1991 (1991-11-26)	1-14
X	claims 1-7	15-37
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Y	LI W-J ET AL: "ELECTROSPUN NANOFIBROUS STRUCTURE: A NOVEL SCAFFOLD FOR TISSUE ENGINEERING" JOURNAL OF BIOMEDICAL MATERIALS RESEARCH, WILEY, NEW YORK, NY, US, vol. 60, no. 4, 15 June 2002 (2002-06-15), pages 613-621, XP001183765 ISSN: 0021-9304	11,12
X	abstract . page 615, column 1, last paragraph - column 2, paragraph 3 Results and Discussion	15,16, 18-21, 23,38
Y	SHIN MICHAEL ET AL: "In vivo bone tissue engineering using mesenchymal stem cells on a novel electrospun nanofibrous scaffold." TISSUE ENGINEERING. 2004 JAN-FEB, vol. 10, no. 1-2, January 2004 (2004-01), pages 33-41, XP002389116 ISSN: 1076-3279	11,12
X	abstract the whole document	15,16, 18-21, 23,38

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LI W-J ET AL: "A three-dimensional nanofibrous scaffold for cartilage tissue engineering using human mesenchymal stem cells" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 26, no. 6, February 2005 (2005-02), pages 599-609, XP004523985 ISSN: 0142-9612	11,12
X	the whole document	15,16, 18-21, 23,38

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 1-14 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.2

Claims Nos.: -

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty of claims 15-37. So many documents were retrieved that it is impossible to determine which parts of the claims 15-37 may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, the search was performed taking into consideration the non-compliance in determining the extent of the search of claims 15-37 .

The search of claims 15-37 was therefore restricted to the scaffolds explicitly mentioned in the examples as well as to the overall concept underlying the application.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2006/000837

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-14 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2006/000837

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