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(56) Documents Cited:

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(58) Field of Search:
INT CL **A61L, C08F, C08G, C08L**
Other: **EPODOC & WPI**

(54) Title of the Invention: **Biocompatible materials**
Abstract Title: **Biocompatible materials**

(57) A fibrous tissue scaffold wherein fibres of the scaffold comprise a phosphonic acid polymer. The fibres preferably incorporate a vinylphosphonic acid — acrylic acid copolymer, and may further include polycaprolactone. There is further described a medical implant coating comprising a phosphonic acid polymer, a biocompatible fibre comprising a phosphonic acid polymer and a biocompatible polymer comprising polycaprolactone and a phosphonic acid polymer.

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Figure 1a

Figure 1b

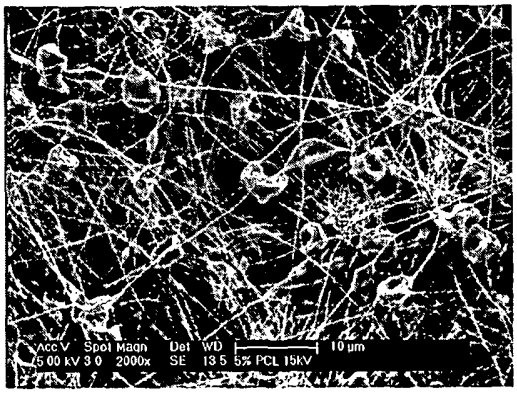


Figure 2a

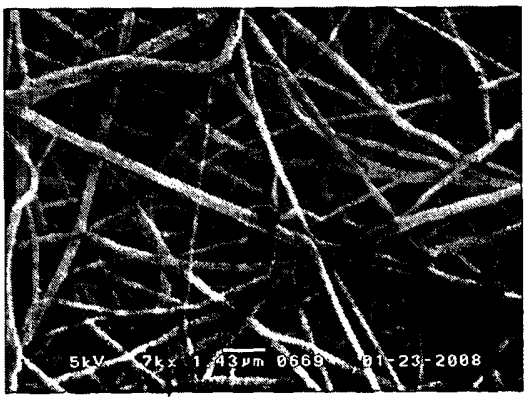


Figure 2b

Figure 3a

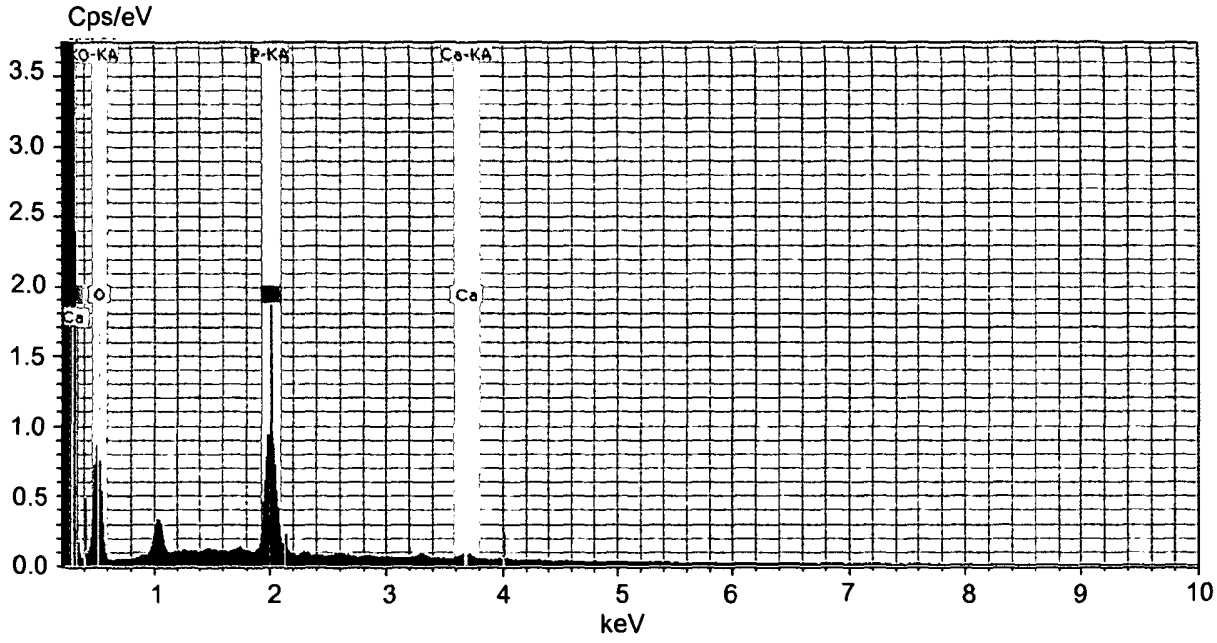


Figure 3b

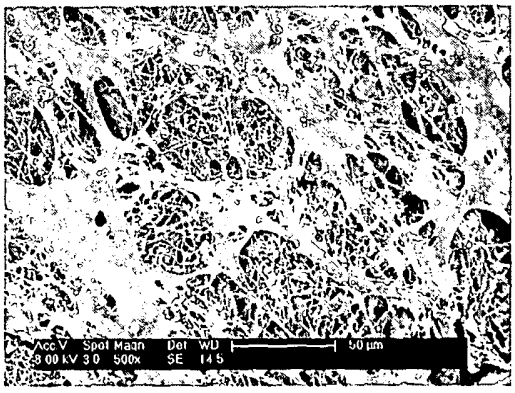


Figure 4a

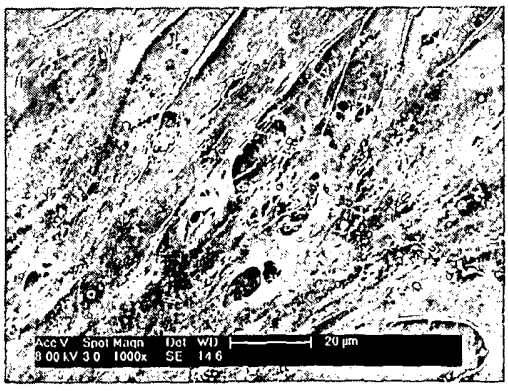
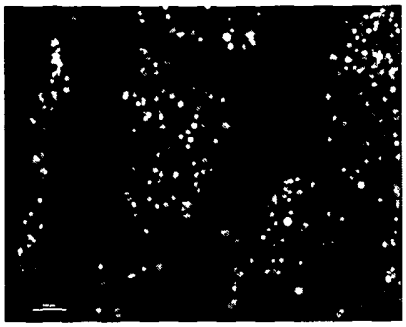


Figure 4b

Inventive Scaffold

Live cells



2 hours

Dead Cells

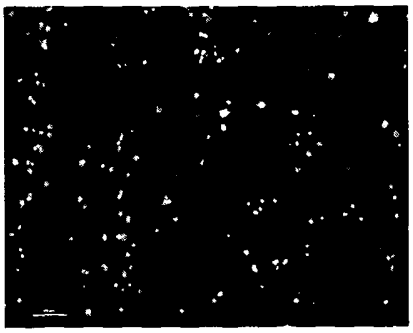


Figure 5a

PCL

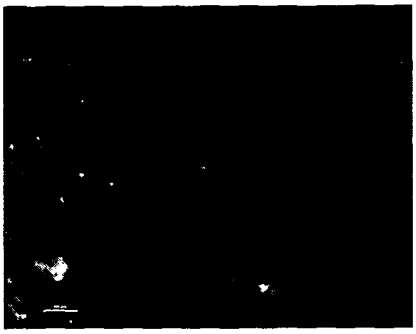
Live Cells

Dead Cells

Figure 5b

Inventive Scaffold

Live Cells



24 hours

Dead Cells

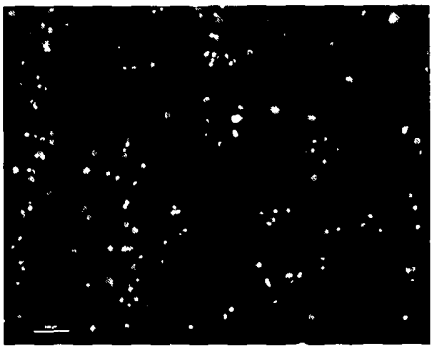


Figure 5c

PCL

Live Cells

Dead Cells

Figure 5d

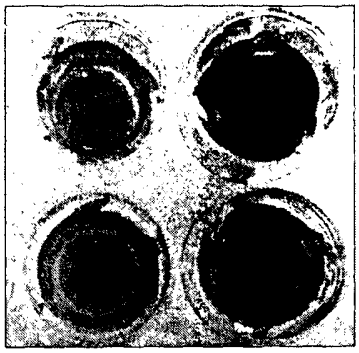


Figure 6

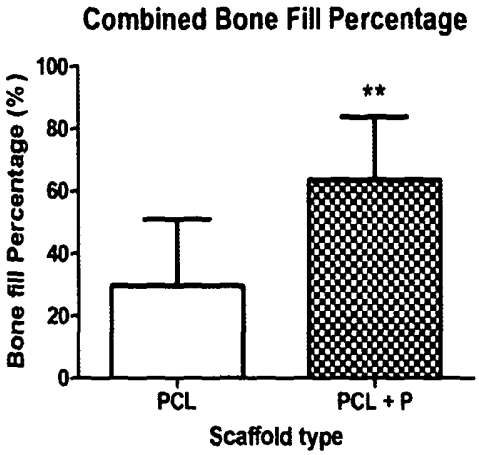


Figure 8

Inventive Scaffold

PCL

Week 1

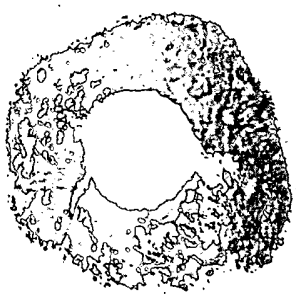


Figure 7a

Figure 7b

Week 6

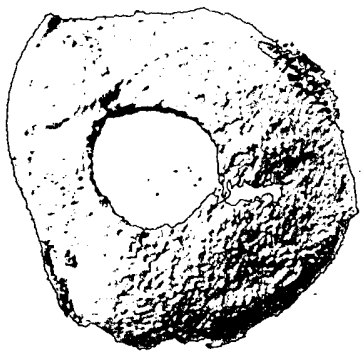


Figure 7c

Figure 7d

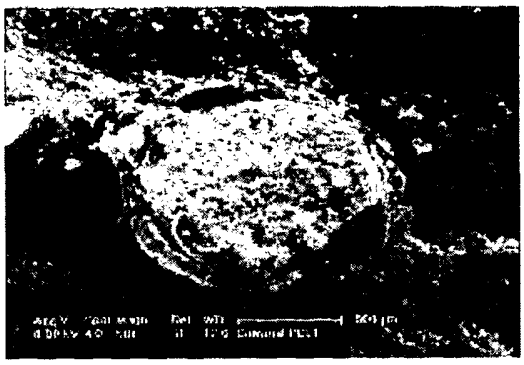


Figure 9a

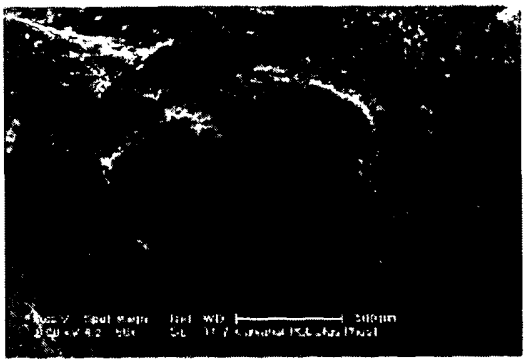


Figure 9b

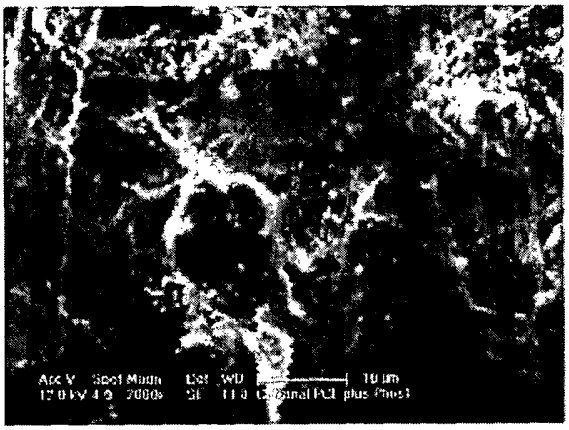


Figure 10

Figure 11

BIOCOMPATIBLE MATERIALS

The present invention relates to biocompatible materials, such as tissue scaffolds in the form of beads, films and the like, and coatings for medical implants.

Biocompatible materials, such as bone graft substitutes, produced by synthetic means are becoming ever more important as new products and methods are sort to treat medical conditions resulting from experiences ranging from acute trauma (e.g. fractures of the bone or tooth due to accident) to chronic illness (e.g. osteoporosis or tooth decay).

In healthy individuals relatively minor fractures to bone resulting from acute trauma usually heal naturally over a short period of time. In more severe cases, a bone graft or implant of some kind may be required. However in osteoporotic patients the risk of initial fracture is greatly increased as compared to a healthy individual and the ability for the individual to recover naturally is significantly reduced. There is a clinical need for a product(s) as bone graft substitutes which could be used clinically for patients with osteoporosis who suffer a fracture and/or require a bone filler in conjunction with a joint replacement.

Osteoporosis is a disease of the bone in which bone mineral density is lower than normal and the microarchitecture and protein structure of the bone is disrupted as a result of homeostatic imbalance between bone formation by cells known as osteoblasts and bone resorption by cells known as osteoclasts.

Osteoporosis is a chronic illness that affects an estimated 75 million people worldwide and there were an estimated 9 million osteoporotic fractures in 2000. Twenty-four percent of women and 33 % of men suffering from osteoporosis die within one year of suffering a hip fracture. As Europe's population ages, the number of people affected by osteoporosis is set to rise significantly; indications suggest that hip fractures alone are expected to double in the next 50 years. The total direct cost of osteoporosis in Europe is estimated at £21 billion and is expected to increase to £51 billion by 2050.

Paget's disease (osteitis deformans) is a chronic illness which is similar to osteoporosis in so far as it also results from an imbalance between bone formation

and bone resorption. As a result, there are similarities between the treatments administered to patients suffering from the two conditions.

Currently, osteoporotic patients and Paget's disease patients are administered a group of drugs called bisphosphonates which are taken orally. Bisphosphonates act by binding to hydroxyapatite in bone; consequently the drug is internalised by osteoclasts and this leads to osteoclast apoptosis. Unfortunately, patients being administered bisphosphonates can suffer from very undesirable side-effects, exhibiting, for example, fever and flu like symptoms. There is also no commercially available implant designed for patients suffering from osteoporosis and/or Paget's disease, or that can be implanted at a specific site where it is needed.

An object of the present invention is to obviate or mitigate one or more of the aforementioned problems and/or to provide improved biocompatible materials, such as bone graft substitutes, designed for tissue regeneration and/or repair.

According to a first aspect of the present invention there is provided a fibrous tissue scaffold comprising a phosphonic acid polymer.

The first aspect of the present invention relates to a scaffold (i.e. a two or three dimensional supporting structure) for tissue attachment and/or growth in which the scaffold structure comprises a phosphonic acid polymer. The phosphonic acid polymer may be incorporated into the material of the fibres of the scaffold and/or provided as a coating over at least a region of the scaffold. Thus, some or all of the fibres of the scaffold may be formed from a phosphonic acid polymer and/or some or all of the fibres of the scaffold may be provided with a coating containing a phosphonic acid polymer. By way of example, fibres of the scaffold may be formed from one or more types of phosphonic acid polymer, and/or the coating may incorporate one or more types of phosphonic acid polymer. Moreover, the fibres may be formed from one type of phosphonic acid polymer and the coating may contain a different type of phosphonic acid polymer. In addition, phosphonic acid moieties may be formed by means of chemical reaction on the surface of the scaffold or by functionalisation of the scaffold with an appropriate molecule containing phosphonic acid moieties.

Reference herein to a "phosphonic acid polymer" encompasses any type of polymer produced by polymerisation of a monomer incorporating a phosphonic acid group and

a polymerisable moiety (e.g. a vinyl group), optionally in combination with one or more other types of monomer. It will also be appreciated that reference herein to a "phosphonic acid polymer" also encompasses polymers of phosphonic acid salts. Moreover, the aforementioned polymers may comprise at least one phosphono or phosphino moiety.

Since the scaffold comprises a phosphonic acid polymer with phosphorus as a pendant group on the backbone, which is analogous to the active moiety in bisphosphonates, the scaffold possesses the ability to bind to the predominant constituent of bone and teeth, hydroxyapatite, and to be internalised by resorbing osteoclasts. Chemical analysis of a scaffold in accordance with the first aspect of the present invention, described in more detail in the Examples, has indicated that calcium is concentrated in areas where there is an increased phosphorous concentration suggesting that there is binding between phosphorous and calcium. *In vitro* studies have demonstrated cellular interactions with the scaffold leading to cell attachment and proliferation and the formation of extracellular matrix. Preferred embodiments of the scaffold possess a porous structure which allows the migration of cells throughout and therefore further increases the area accessible for cellular interactions.

In light of the above, the tissue engineered scaffold of the first aspect of the present invention is therefore eminently suitable for use by patients suffering from osteoporosis, Paget's disease and/or chronic dental illness to improve bone mass and/or bone stock at areas where it is most needed. It is also anticipated that since the phosphonic acid polymer-containing scaffold contains only the active moiety of the bisphosphonates family of compounds (i.e. the phosphorous-carbon bond), the scaffold of the first aspect of the present invention can be considered as a non-pharmaceutical product and should eliminate some, if not all, of the unwanted side effects currently associated with conventional bisphosphonate treatment.

A still further advantage of the scaffold of the first aspect of the present invention is that in preferred embodiments it is biodegradable therefore eliminating the need for further invasive surgery consequently reducing hospital expenses and risk of infection.

The skilled person will understand that the scaffold is suitable for wider application in the repair and regeneration of bone and teeth in otherwise healthy individuals. For

example, in the treatment of individuals who have suffered some form of acute trauma to bone or tooth.

A first preferred embodiment of the present invention provides a fibrous tissue scaffold wherein fibres of the scaffold are formed from a phosphonic acid polymer.

The first preferred embodiment thus relates to a scaffold in which the material from which the structure is produced incorporates a phosphonic acid polymer. That is, the phosphonic acid polymer forms an integral part of the scaffold material as distinct from having been applied in some form of surface treatment process to provide a phosphonic acid surface coating to the scaffold. It will be appreciated, however, that the scaffold can be provided with any desirable type of surface coating, including a coating incorporating a phosphonic acid-based compound or polymer to modify or enhance the chemical, biological and/or physical properties of the scaffold incorporating phosphonic acid polymer-based fibres.

Moreover, the scaffold of the first preferred embodiment of the present invention may incorporate fibres made exclusively from a phosphonic acid polymer or the scaffold may incorporate a combination of different types of fibres wherein some, but not all, of the fibres making up the scaffold are formed from a phosphonic acid polymer.

A second preferred embodiment provides a fibrous tissue scaffold wherein fibres of the scaffold are provided with a coating containing a phosphonic acid polymer.

The phosphonic acid polymer-containing coating may be applied to substantially all of the fibres making up the scaffold or just a portion thereof. Moreover, the or each fibre of the scaffold provided with such a coating may be substantially completely covered with the coating or the coating may be applied to just a portion or region of the or each fibre. The coating may cover from around 0.00001 to 100 % of the fibre's surface area, more preferably around 0.001 to 100 % of the fibre's surface area, and still more preferably around 0.1 to 100 % of the fibre's surface area. Moreover, the coating may be one layer thick or may be up to thousands of layers thick depending upon the intended application of the coated fibres.

The starting material that is formed into the fibres of the scaffold according to the first preferred embodiment of the present invention may be a phosphonic acid oligomer, homopolymer or heteropolymer (e.g. copolymer, terpolymer, etc), or may be a

mixture or blend of any such phosphonic acid moieties in combination with one or more other polymers. The molecular weight of the polymer could be that of the combination of a few monomer units forming the polymer (as defined by the regulatory bodies as "polymer") to several million depending upon the intended application of the polymer. More preferably the molecular weight is in the range of around 100 g/mol to 500,000 g/mol, and most preferably the molecular weight is in the range of around 100 g/mol to 200,000 g/mol.

In a preferred embodiment the fibres of the scaffold are formed from a homopolymer of a phosphonic acid monomer, such as vinyl phosphonic acid (VPA). Alternatively, the fibres of the scaffold may be formed from a co-polymer of a phosphonic acid monomer copolymerised with a second type of polymerisable monomer. Preferred copolymerisable monomers include polymerisable carboxylic acid monomers, such as acrylic acid and methacrylic acid (referred to herein generically as "(meth)acrylic acid") monomers.

In accordance with preferred embodiments of the present invention, the polymer may incorporate an active moiety selected from a phosphono-component and/or phosphino-component. The phosphono-component or phosphino-component may consist essentially of vinylphosphonic acid (VPA), vinylidene-1,1-diphosphonic acid (VDPA), a phosphono substituted mono- or di-carboxylic acid, hypophosphorus acid or a salt, such as an alkali metal salt of hypophosphorus acid. By way of example, the phosphono-component may consist essentially of a homopolymer of VPA, VDPA, or phosphono-succinic acid.

A composition incorporating the polymer may further include one or more additional components, such as unsaturated sulphonic acid, saturated or unsaturated carboxylic acids, unsaturated amides, primary or secondary amines, polyalkylene imines, or amine-terminated polyalkylene glycols. For example, the polymeric composition may consist essentially of a copolymer of vinylphosphonic acid (VPA) with vinylsulphonic acid (VSA), or with acrylic acid (AA), methacrylic acid (MAA) or acrylamide. Alternatively, the polymeric composition may consist essentially of a copolymer of VDPA with VSA, or with AA, MAA or acrylamide. As another example, the polymeric composition may consist essentially of a terpolymer of VDPA, VSA and either AA, MMA or acrylamide. Alternatively, the polymeric composition may consist essentially of the reaction product VPA, or VDPA, or a mixture of VPA and VDPA, and any one of the following:

a. a primary amine;

b. a secondary amine;

c. a polyethylene imine;

d. an amine terminated polyethylene or polypropylene glycol (e.g. jeffamines); and/or

e. a hypophosphorus acid or salt thereof.

A particularly preferred copolymer for use in fabricating fibres for the scaffold is poly (vinyl phosphonic acid -co- acrylic acid).

It will be appreciated that any of the aforementioned polymers, copolymers or terpolymers may comprise at least one phosphono or phosphino moiety.

The scaffold according to the first aspect of the present invention is preferably formed from one or more biocompatible polymers. Preferably fibres of the scaffold are made from or comprise polycaprolactone. Polycaprolactone (poly-ε-caprolactone) is a biocompatible material which is approved by the US Food and Drug Administration (FDA) for use in biomedical applications. It is thus eminently suitable for application in tissue scaffolds according to the first aspect of the present invention. In accordance with the present invention, other biocompatible polymers can be used in addition to, or in place of, polycaprolactone, such as poly(glycolic acid), poly(lactic acid), poly(lactic-co-glycolic acid), and/or poly(methyl methacrylate).

In a particularly preferred embodiment of the first aspect of the present invention there is provided a fibrous tissue scaffold wherein at least some of the fibres are formed from a polymer solution comprising polycaprolactone and poly (vinyl phosphonic acid -co- acrylic acid).

The scaffold according to the first aspect of the present invention preferably comprises sub-micron diameter fibres. The fibres may possess an average diameter in the range of around 10 to 1000 nm, more preferably around 100 to 500 nm, and yet more preferably around 200 to 400 nm. It is particularly preferred that the diameters possess an average diameter of around 250 to 300 nm.

The fibres within the scaffold may have a substantially ordered arrangement, partly ordered arrangement or be essentially randomly arranged. It is particularly preferred

that the fibres are randomly arranged since this most closely mirrors the physical structure of the natural biological environment in which the scaffold is intended to be employed.

The scaffold may be provided in the form of a three dimensional bead or packing material of any desirable size and shape, or may be provided as an essentially two dimensional sheet or strip of fibrous material. It will, of course, be appreciated that even such "two dimensional" materials will possess a third dimension, e.g. thickness, but that materials of this kind possess two predominant dimensions, e.g. width and length.

In a preferred embodiment the scaffold according to the first aspect of the present invention is provided in the form of a polymeric packing material for insertion into a bone or tooth cavity. It will be appreciated that it is important that the packing materials is capable of withstanding the levels of compressive forces experienced by the implant site within the body. While the packing material may be of any desirable size and/or shape, it is preferred that the packing material is in the form of a pellet.

In a further preferred embodiment the scaffold is provided in the form of a polymeric sheet, which can exhibit any desirable degree of flexibility to suit an intended site of implantation.

The scaffold is preferably porous, which is advantageous since it allows infiltration of cells throughout the structure of the scaffold and affords a significantly increased specific surface area for cell growth as compared to non-porous materials. Porous scaffolds also facilitate the penetration of nutrients from cell culture media through the scaffold to the growing cells supported by the scaffold. Additionally, providing the scaffold with a porous structure affords a means to manipulate and control various physical, chemical and biological properties of the scaffold so that they can be tailored to suit a particular application.

A particularly preferred method for producing the phosphonic acid-based fibres in the scaffold of the present invention is electrospinning a solution of a suitable phosphonic acid polymer, or polymer mixture or blend containing a suitable phosphonic acid polymer.

During the electrospinning process a polymer is fed through a needle at a constant rate and a voltage is applied. The voltage leads to the formation of a Taylor's cone at the needle tip and when the electrostatic forces overcome the surface tension of the polymer solution, a polymer jet is formed which is attracted to a grounded collector plate.

The feed rate of the polymer may be altered to control the nature of the fibres formed and to take account of the properties of the particular polymer being spun. It is preferred that feed rate is around 0.01 to 0.1 ml/min, more preferably around 0.05 ml/min. The applied voltage may be varied according to the properties of the particular polymer being electrospun and/or the desired properties of the final fibres. The applied voltage may be around 5 to 40 kV, more preferably around 10 to 30 kV and is most preferably around 20 kV. The separation of the needle from the collector plate is a further parameter which can be changed to control the nature of the final fibres depending upon the polymer being used. The needle may be spaced around 10 to 20 cm from the collector plate, and is preferably spaced around 15 cm from the collector plate.

In order to fabricate a scaffold comprised primarily of well-defined fibres, rather than non-fibrous agglomerations of polymer, it is preferred that the electrospinning solution should contain at least around 6 w/v% of the polymer, more preferably around 7 to 15 w/v% of the polymer, and most preferably around 10 w/v% of the polymer.

The polymer solution being electrospun may incorporate any appropriate solvent. A preferred solvent is acetone.

As described above, a preferred embodiment of the fibrous scaffold according to the first aspect of the present invention contains fibres made from a polymer mixture containing a vinyl phosphonic acid / acrylic acid copolymer and polycaprolactone. In this case, it is preferred that the following electrospinning parameters are adopted: 10 w/v% polycaprolactone in acetone; applied voltage of 20 kV; polymer flow rate of 0.05 ml/min; and a needle / collector distance of 15 cm.

A second aspect of the present invention provides a biocompatible fibre comprising a phosphonic acid polymer.

The biocompatible fibre according to the second aspect of the present invention may incorporate a phosphonic acid polymer as a coating and/or within the structure of the fibre as described above in relation to the first aspect of the present invention. The fibre according to the second aspect may therefore possess any of the physical, chemical and/or biological properties set out above in respect of the phosphonic acid polymer-containing fibres employed in the scaffold according to the first aspect of the present invention.

A third aspect of the present invention provides a medical implant coating comprising a phosphonic acid polymer.

A fourth aspect of the present invention provides medical implant provided with a coating comprising a phosphonic acid polymer.

The coating of the third and fourth aspects may comprise one or more types of phosphonic acid polymer, optionally in combination with one or more further polymers. The coating may be a phosphonic acid homopolymer or heteropolymer, or may be a mixture or blend of any such phosphonic acid polymer in combination with one or more other polymers. The molecular weight of the polymer could be that of the combination of a few monomer units forming the polymer (as defined by the regulatory bodies as "polymer") to several million depending upon the intended application of the polymer. More preferably the molecular weight is in the range of around 100 g/mol to 500,000 g/mol, and most preferably the molecular weight is in the range of around 100 g/mol to 200,000 g/mol.

In a preferred embodiment the coating comprises a homopolymer of a phosphonic acid monomer, such as vinyl phosphonic acid (VPA). In another embodiment, the coating is a co-polymer of a phosphonic acid monomer copolymerised with a second type of polymerisable monomer, such as a polymerisable carboxylic acid monomer. Preferred examples of copolymerisable monomers include (meth)acrylic acid monomers.

In accordance with preferred embodiments of the present invention, the polymer may incorporate an active moiety selected from a phosphono-component and/or phosphino-component. The phosphono-component or phosphino-component may consist essentially of vinylphosphonic acid (VPA), vinylidene-1,1-diphosphonic acid (VDPA), a phosphono substituted mono- or di-carboxylic acid, hypophosphorus acid

or a salt, such as an alkali metal salt of hypophosphorus acid. By way of example, the phosphono-component may consist essentially of a homopolymer of VPA, VDPA, or phosphono-succinic acid.

A composition incorporating the polymer may further include one or more additional components, such as unsaturated sulphonic acid, saturated or unsaturated carboxylic acids, unsaturated amides, primary or secondary amines, polyalkylene imines, or amine-terminated polyalkylene glycols. For example, the polymeric composition may consist essentially of a copolymer of vinylphosphonic acid (VPA) with vinylsulphonic acid (VSA), or with acrylic acid (AA), methacrylic acid (MAA) or acrylamide. Alternatively, the polymeric composition may consist essentially of a copolymer of VDPA with VSA, or with AA, MAA or acrylamide. As another example, the polymeric composition may consist essentially of a terpolymer of VDPA, VSA and either AA, MMA or acrylamide. Alternatively, the polymeric composition may consist essentially of the reaction product VPA, or VDPA, or a mixture of VPA and VDPA, and any one of the following:

a. a primary amine;
b. a secondary amine;
c. a polyethylene imine;
d. an amine terminated polyethylene or polypropylene glycol (e.g. jeffamines); and/or
e. a hypophosphorus acid or salt thereof.

A particularly preferred copolymer for use in the coating is poly (vinyl phosphonic acid -co- acrylic acid).

The coating preferably comprises one or more biocompatible polymers in addition to the phosphonic acid polymer(s), such as polycaprolactone (poly-ε-caprolactone). In accordance with the present invention, other biocompatible polymers can be used such as poly(glycolic acid), poly(lactic acid), poly(lactic-co-glycolic acid), poly(methyl methacrylate).

In a particularly preferred embodiment of the third and/or fourth aspects of the present invention there is provided a medical implant coating comprising polycaprolactone and poly (vinyl phosphonic acid -co- acrylic acid).

It is envisaged that the coating may be applied to any desirable medical implant. The coating is perceived as being particularly suitable for use in applications in which a surface of the medical implant will contact adjacent areas of bone and/or tooth. It is therefore preferred that the surface(s) of the medical implant to which the coating is intended to be applied or has been applied is that surface or are those surfaces which will contact bone and/or tooth following implantation. Medical implant devices may be fabricated from a range of different biocompatible materials, a common one of which is titanium. Example 8 below describes the successful application of a polycaprolactone / poly(vinyl phosphonic acid –co- acrylic acid) polymer coating to a titanium substrate in which after only three days significant levels of osteoblast attachment were observed thereby demonstrating the suitability of this coating for application as a medical coating at the implant / bone interface of the implant.

A fifth aspect of the present invention provides biocompatible polymer composition comprising polycaprolactone and a phosphonic acid polymer.

The polymer composition may be a mixture, blend or heteropolymer incorporating copolymerised phosphonic acid and caprolactone monomers, optionally including one or more further monomers. Where the polymer composition is a mixture or blend of a phosphonic acid component and a polycaprolactone component, the phosphonic acid component may be a phosphonic acid homopolymer or heteropolymer. The molecular weight of the polymer could be that of the combination of a few monomer units forming the polymer (as defined by the regulatory bodies as "polymer") to several million depending upon the intended application of the polymer. More preferably the molecular weight is in the range of around 100 g/mol to 500,000 g/mol, and most preferably the molecular weight is in the range of around 100 g/mol to 200,000 g/mol.

In a preferred embodiment the phosphonic acid component of the polymer composition comprises a homopolymer of a phosphonic acid monomer, such as vinyl phosphonic acid (VPA). In another embodiment, the phosphonic acid component is a co-polymer of a phosphonic acid monomer copolymerised with a second type of polymerisable monomer, such as a polymerisable carboxylic acid monomer, for example, a (meth)acrylic acid monomer.

In accordance with preferred embodiments of the present invention, the polymer may incorporate an active moiety selected from a phosphono-component and/or

phosphino-component. The phosphono-component or phosphino-component may consist essentially of vinylphosphonic acid (VPA), vinylidene-1,1-diphosphonic acid (VDPA), a phosphono substituted mono- or di-carboxylic acid, hypophosphorus acid or a salt, such as an alkali metal salt of hypophosphorus acid. By way of example, the phosphono-component may consist essentially of a homopolymer of VPA, VDPA, or phosphono-succinic acid.

A composition incorporating the polymer may further include one or more additional components, such as unsaturated sulphonic acid, saturated or unsaturated carboxylic acids, unsaturated amides, primary or secondary amines, polyalkylene imines, or amine-terminated polyalkylene glycols. For example, the polymeric composition may consist essentially of a copolymer of vinylphosphonic acid (VPA) with vinylsulphonic acid (VSA), or with acrylic acid (AA), methacrylic acid (MAA) or acrylamide. Alternatively, the polymeric composition may consist essentially of a copolymer of VDPA with VSA, or with AA, MAA or acrylamide. As another example, the polymeric composition may consist essentially of a terpolymer of VDPA, VSA and either AA, MMA or acrylamide. Alternatively, the polymeric composition may consist essentially of the reaction product VPA, or VDPA, or a mixture of VPA and VDPA, and any one of the following:

a. a primary amine;
b. a secondary amine;
c. a polyethylene imine;
d. an amine terminated polyethylene or polypropylene glycol (e.g. jeffamines); and/or
e. a hypophosphorus acid or salt thereof.

A particularly preferred copolymer for use in the phosphonic acid component of the polymer composition is poly (vinyl phosphonic acid -co- acrylic acid). Thus, a particularly preferred embodiment of the fifth aspect of the present invention provides a polymer composition comprising a mixture of polycaprolactone and poly (vinyl phosphonic acid -co- acrylic acid).

Polymer compositions according to the fifth aspect of the present invention are eminently suitable for application as coatings on medical implants as mentioned above in respect of the third and fourth aspects of the present invention.

A sixth aspect of the present invention provides a method of culturing mammalian tissue cells, the method comprising culturing mammalian tissue cells on a fibrous tissue scaffold, fibres of the scaffold being comprising a phosphonic acid polymer, and exposing said cells to a cell culture medium to facilitate adherence of said cells to the scaffold.

A seventh aspect of the present invention provides a method of repairing or regenerating mammalian tissue comprising culturing mammalian tissue cells on a fibrous tissue scaffold, fibres of the scaffold comprising a phosphonic acid polymer, exposing said cells to a cell culture medium to facilitate adherence of said cells to the scaffold, and placing the scaffold with cells adhered thereto adjacent to the mammalian tissue in need of repair or regeneration.

It will be appreciated that the scaffold employed in the sixth and/or seventh aspects of the present invention may comprise any of the features set out in the preferred embodiments of the scaffold according to the first aspect of the present invention. Moreover, the scaffold of the sixth and/or seventh aspects may incorporate biocompatible fibres according to the second aspect of the invention.

Preferred embodiments of aspects of the present invention will now be demonstrated with reference to the following non-limiting examples, in which:

Figures 1a and 1b are scanning electron microscope (SEM) images of healthy bone and osteoporotic bone respectively;

Figures 2a and 2b are scanning electron microscope (SEM) images of fibrous materials formed by electrospinning polymer solutions containing 5 % and 10 % polycaprolactone (PCL) respectively;

Figure 3a is a colour-coded image of the internal structure of a tissue scaffold according to the first aspect of the present invention generated from energy dispersive x-ray (EDX) analysis of the scaffold

Figure 3b is an energy dispersive x-ray (EDX) spectrum for the material shown in Figure 3a;

Figures 4a and 4b are scanning electron microscope (SEM) images at 500x and 1000x magnification respectively of human osteoblasts adhered to a tissue scaffold according to the first aspect of the present invention;

Figures 5a to 5d are colour-coded images of live (green) and dead (red) cells adhered to a tissue scaffold according to the first aspect of the present invention (Figures 5a and 5c) and a tissue scaffold made from polycaprolactone (PCL) acting as a control (Figures 5b and 5d). Figures 5a and 5b are images taken after 2hrs and Figures 5c and 5d are images taken after 24 hours;

Figure 6 is a photograph of tissue scaffolds analysed using Alizarin red staining to determine levels of calcium ion binding to each scaffold. The tissue scaffolds on the left were made from PCL to act as a control and the tissue scaffolds on right were in accordance with the first aspect of the present invention;

Figures 7a to 7d are microscopic x-ray computed tomography (micro-CT) images of calvarial samples provided with critical sized (1.5 mm diameter) defects and subsequently treated with a tissue scaffold according to the first aspect of the present invention (Figures 7a and 7c) or a control tissue scaffold made from polycaprolactone (PCL) (Figures 7b and 7d). Figures 7a and 7b are images taken after one week and Figures 7c and 7d are images taken after 6 weeks;

Figure 8 is a graph showing the level of bone formation observed for the tissue scaffold according to the first aspect of the present invention illustrated in Figure 7c and the control tissue scaffold made from PCL illustrated in Figure 7d;

Figures 9a and 9b are scanning electron microscopy (SEM) images of calvarial samples provided with critical sized (1.5 mm diameter) defects and subsequently treated with a control tissue scaffold made from polycaprolactone (PCL) (Figure 9a) and a tissue scaffold according to the first aspect of the present invention (Figure 9b). Images taken 6 weeks after implantation;

Figure 10 is a scanning electron microscopy (SEM) image of a scaffold according to the first aspect of the present invention after implantation showing hydroxyapatite-like formations; and

Figure 11 is a scanning electron microscopy (SEM) image of a surface of a titanium substrate coated with a polymeric coating according to the second aspect of the present invention showing osteoblast attachment and formation.

EXAMPLES

Example 1

Fabrication of Scaffold

Various electrospinning parameters were investigated to determine which would be best for our application. Different concentrations of polycaprolactone were used varying from 2% to 20%, and the voltages applied were varied to achieve optimum conditions.

Polycaprolactone fibres were fabricated by an electrospinning process. Acetone was used as a solvent to dissolve polycaprolactone pellets under gentle stirring and heat to obtain a polycaprolactone concentration of 10 w/v%.

The polymer solution was placed in a 10 ml syringe fitted to a blunted needle with a diameter of 0.8 mm. A high voltage power supply was attached to the needle and a voltage of 20 kV was applied. A grounded collector plate was located at a fixed distance of 15 cm from the needle tip.

A syringe pump was used to feed the polycaprolactone polymer through the needle at a constant rate of 0.05ml/min. A taylor cone was formed when the voltage was applied and this led to the formation of a polymer jet which was attracted to the grounded collector plate.

Scanning electron microscopy (SEM) analysis demonstrated that lower PCL concentrations of 2 w/v% and 5 w/v% gave rise to beads within the fibrous mat (see figure 2a) whereas a higher PCL concentration, for example a PCL concentration of 10 w/v%, yielded clearly defined fibres with an average fibre diameter of 266 nm (see figure 2b). Beaded fibrous structures are undesirable for the aforementioned application. Concentrations of PCL much higher than 10 w/v%, for example, above 15 w/v% are not amenable to electrospinning due to the viscosity of their solutions.

Example 2

Calcium Ion Adsorption to Scaffold

Scaffolds according to the first aspect of the present invention containing phosphonic acid polymer were immersed in a calcium rich solution for three days. After 3 days, energy dispersive x-ray (EDX) analysis demonstrated an increase in calcium ions at areas of high phosphorous concentration (see figure 3a). Calcium phosphate formation is vital for bone formation and *in vitro* aggregation of the two elements is promising for *in vivo* bone formation. The presence of phosphorous and calcium is confirmed in the EDX spectrum as shown in figure 3b. The spectrum also confirms that phosphorous was still present after 3 days of immersion in a liquid.

Example 3

Cell Growth on Scaffold

Human osteoblasts have been shown to attach and proliferate on scaffolds according to the first aspect of the present invention as shown in figure 4. The cells were observed to have spread over the surface of the scaffolds and displayed normal osteoblast structure.

Example 4

Cell Survival on Scaffold

Live/dead staining distinguishes live cells (visible as green) from dead cells (visible as red). After 2 hours, cells on both a control scaffold (pure PCL) and a scaffold according to the first aspect of the present invention containing phosphonic acid polymer are rounded as would be expected. There appeared to be more live cells on the scaffold containing the phosphonic acid polymer as shown in figure 5. On the pure PCL scaffold there were fewer live cells, and more dead cells. After 24 hours the cells were observed to have spread across the surfaces of the scaffolds and, as with the 2 hour time point, there were fewer dead cells on the scaffold containing the phosphonic acid polymer according to the first aspect of the present invention.

Example 5

Mineralising Capacity of Scaffold – Alizarin Staining

Human osteoblasts were seeded on to two control scaffolds (pure PCL) and two scaffolds according to the first aspect of the present invention. Alizarin red staining to identify the presence of calcium ions was carried out after 3 weeks. It was observed that the scaffolds containing the phosphonic acid polymer were stained a deep red colour, whereas the pure PCL scaffolds only had specks of red as shown in figure 6.

This result suggests that calcium apatite had formed much more rapidly on the scaffolds containing the phosphonic acid polymer according to the first aspect of the present invention.

Example 6

Mineralising Capacity of Scaffold – Bone Mineral Volume

The calvarial from 4 day old mice were extracted aseptically and a 1.5 mm diameter critical sized defect was created, which under normal circumstances would not heal. A scaffold containing phosphonic acid polymer according to the first aspect of the present invention was added to one such defect and a control scaffold (pure PCL) added to another defect. A total of five pairs of samples were prepared in this way for testing. After 6 weeks of culture (osteogenic media – DMEM supplemented with 10 % FBS, 100 µg / ml penicillin / streptomycin, 0.05 mM ascorbic acid, 0.1 µM dexamethasone, 10 mM β-glycerophosphate disodium salt) there was a significant difference in bone mineral volume between the two samples when examined using microscopic x-ray computed tomography (micro-CT) as shown in figure 7. Scanning electron microscopy (SEM) images of the treated samples also demonstrated that the scaffold according to the first aspect of the present invention displayed better integration into the defect site than the control scaffold (see Figures 9a and 9b).

As very few osteoclasts are present in the calvarial we can assume that the phosphonic acid polymer has an effect on osteoblast activity as well as osteoclast activity. The statistical difference in bone fill percentage over the five pairs of samples is shown in figure 8 from which it can be concluded that the scaffolds according to the first aspect of the present invention yielded significantly increased bone formation as compared to the control scaffolds.

The suitability of the scaffold according to the first aspect of the present invention for use in biomedical applications was further demonstrated by the observation of hydroxyapatite-like formations following culturing.

Example 7

Preparation of Coating Formulation

A solution of poly (vinyl phosphonic acid-co-acrylic acid) [Poly(VPA-co-AA)] containing 10-15 % solid polymer in distilled water was prepared.

Example 8

Application of Coating Formulation to Substrate

A titanium (Ti) substrate was heated to 100 to 150 °C before being immersed in a pre-heated bath of poly(VPA-co-AA). The Ti substrate was then removed from the bath and washed with water. As a result, a surface of the titanium substrate was coated with a polymeric coating of poly(VPA-co-AA) according to a preferred embodiment of the second aspect of the present invention.

After three days, significant osteoblast attachment to the coating was observed as shown in Figure 11.

CLAIMS

1. A fibrous tissue scaffold comprising at least one type of phosphonic acid polymer.

2. A scaffold according to claim 1, wherein fibres of the scaffold are formed from a first type of phosphonic acid polymer.

3. A scaffold according to claim 2, wherein fibres of the scaffold are produced by electrospinning a solution of said first type of phosphonic acid polymer.

4. A scaffold according to any one of claims 1 to 3, wherein said phosphonic acid polymer comprises at least one phosphono or phosphino moiety.

5. A scaffold according to any one of claims 1 to 3, wherein said phosphonic acid polymer is a phosphonic acid homopolymer, or a phosphonic acid heteropolymer incorporating one or more other types of polymer.

6. A scaffold according to any one of claims 1 to 3, wherein said phosphonic acid polymer is a phosphonic acid homopolymer selected from the group consisting of vinyl phosphonic acid, vinylidene-1,1-diphosphonic acid, phosphono-substituted mono-carboxylic acid, phosphono-substituted di-carboxylic acid, hypophosphorus acid, a hypophosphorus acid salt, and phosphono-succinic acid.

7. A scaffold according to any one of claims 1 to 3, wherein said phosphonic acid polymer is a phosphonic acid heteropolymer of phosphonic acid and one or more further monomers selected from the group consisting of carboxylic acid, sulphonic acid, amides, amines, polyalkylene imines, and amine-terminated polyalkylene glycols.

8. A scaffold according to any one of claims 1 to 3, wherein said phosphonic acid polymer is a phosphonic acid heteropolymer selected from the group consisting of vinylphosphonic acid and vinylsulphonic acid, vinylphosphonic acid and acrylic acid, vinylphosphonic acid and methacrylic acid, vinylphosphonic acid and acrylamide, vinylidene-1,1-diphosphonic acid and vinylsulphonic acid, vinylidene-1,1-diphosphonic acid and acrylic acid,

vinylidene-1,1-diphosphonic acid and methacrylic acid, vinylidene-1,1-diphosphonic acid and acrylamide, vinylidene-1,1-diphosphonic acid, vinylsulphonic acid and acrylic acid, vinylidene-1,1-diphosphonic acid, vinylsulphonic acid and methacrylic acid, and vinylidene-1,1-diphosphonic acid, vinylsulphonic acid and acrylamide.

9. A scaffold according to any one of claims 1 to 3, wherein said phosphonic acid polymer is poly(vinyl phosphonic acid-co-acrylic acid).

10. A scaffold according to any preceding claim, wherein the molecular weight of the phosphonic acid polymer is in the range of around 100 g/mol to 500,000 g/mol.

11. A scaffold according to any preceding claim, wherein fibres of the scaffold are provided with a coating containing a second type of phosphonic acid polymer.

12. A scaffold according to claim 11, wherein said second type of phosphonic acid polymer comprises at least one phosphono or phosphino moiety.

13. A scaffold according to claim 11, wherein said second type of phosphonic acid polymer is a phosphonic acid homopolymer, or a phosphonic acid heteropolymer incorporating one or more other types of polymer.

14. A scaffold according to claim 11, wherein said second type of phosphonic acid polymer is a phosphonic acid homopolymer selected from the group consisting of vinyl phosphonic acid, vinylidene-1,1-diphosphonic acid, phosphono-substituted mono-carboxylic acid, phosphono-substituted di-carboxylic acid, hypophosphorus acid, a hypophosphorus acid salt, and phosphono-succinic acid.

15. A scaffold according to claim 11, wherein said second type of phosphonic acid polymer is a phosphonic acid heteropolymer of phosphonic acid and one or more further monomers selected from the group consisting of carboxylic acid, sulphonic acid, amides, amines, polyalkylene imines, and amine-terminated polyalkylene glycols.

16. A scaffold according to claim 11, wherein said second type of phosphonic acid polymer is a phosphonic acid heteropolymer selected from the group consisting of vinylphosphonic acid and vinylsulphonic acid, vinylphosphonic acid and acrylic acid, vinylphosphonic acid and methacrylic acid, vinylphosphonic acid and acrylamide, vinylidene-1,1-diphosphonic acid and vinylsulphonic acid, vinylidene-1,1-diphosphonic acid and acrylic acid, vinylidene-1,1-diphosphonic acid and methacrylic acid, vinylidene-1,1-diphosphonic acid and acrylamide, vinylidene-1,1-diphosphonic acid, vinylsulphonic acid and acrylic acid, vinylidene-1,1-diphosphonic acid, vinylsulphonic acid and methacrylic acid, and vinylidene-1,1-diphosphonic acid, vinylsulphonic acid and acrylamide.

17. A scaffold according to claim 11, wherein said second type of phosphonic acid polymer is poly(vinyl phosphonic acid-co-acrylic acid).

18. A scaffold according to any one of claims 11 to 17, wherein the molecular weight of the second type of phosphonic acid polymer is in the range of around 100 g/mol to 500,000 g/mol.

19. A scaffold according to any one of claims 11 to 18, wherein said phosphonic acid polymer incorporated into the fibres of the scaffold and said further phosphonic acid polymer contained in the coating are the same type of phosphonic acid polymer.

20. A scaffold according to any one of claims 11 to 18, wherein said phosphonic acid polymer incorporated into the fibres of the scaffold and said further phosphonic acid polymer contained in the coating are different types of phosphonic acid polymer.

21. A scaffold according to any preceding claim, wherein fibres of the scaffold comprise at least one additional biocompatible polymer selected from the group consisting of polycaprolactone, poly(glycolic acid), poly(lactic acid), poly(lactic-co-glycolic acid), and poly(methyl methacrylate).

22. A scaffold according to any preceding claim, wherein fibres of the scaffold possess a sub-micron average diameter.

23. A scaffold according to any one of claims 1 to 21, wherein fibres of the scaffold possess an average diameter in the range of around 10 to 1000 nm.

24. A scaffold according to any preceding claim, wherein the scaffold is porous.

25. A method for preparing a fibrous tissue scaffold comprising fibres formed from a phosphonic acid polymer, wherein fibres of the scaffold are produced by electrospinning a solution of said phosphonic acid polymer.

26. A method according to claim 25, wherein said phosphonic acid polymer solution is fed through an electrospinning needle at a feed rate of around 0.01 to 0.1 ml/min.

27. A method according to claim 25 or 26, wherein a voltage of around 5 to 40 kV is applied to said phosphonic acid polymer solution during electrospinning.

28. A method according to claim 25, 26 or 27, wherein an electrospinning needle is spaced around 10 to 20 cm from a collector plate.

29. A method according to any one of claims 25 to 28, wherein said polymer solution contains around 7 to 15 w/v% of the phosphonic acid polymer.

30. A medical implant coating comprising a phosphonic acid polymer.

31. A coating according to claim 30, wherein said phosphonic acid polymer comprises at least one phosphono or phosphino moiety.

32. A coating according to claim 30, wherein said phosphonic acid polymer is a phosphonic acid homopolymer, or a phosphonic acid heteropolymer incorporating one or more other types of polymer.

33. A coating according to claim 30, wherein said phosphonic acid polymer is a phosphonic acid homopolymer selected from the group consisting of vinyl phosphonic acid, vinylidene-1,1-diphosphonic acid, phosphono-substituted mono-carboxylic acid, phosphono-substituted di-carboxylic acid, hypophosphorus acid, a hypophosphorus acid salt, and phosphono-succinic acid.

34. A coating according to claim 30, wherein said phosphonic acid polymer is a phosphonic acid heteropolymer of phosphonic acid and one or more further monomers selected from the group consisting of carboxylic acid, sulphonic acid, amides, amines, polyalkylene imines, and amine-terminated polyalkylene glycols.

35. A coating according to claim 30, wherein said phosphonic acid polymer is a phosphonic acid heteropolymer selected from the group consisting of vinylphosphonic acid and vinylsulphonic acid, vinylphosphonic acid and acrylic acid, vinylphosphonic acid and methacrylic acid, vinylphosphonic acid and acrylamide, vinylidene-1,1-diphosphonic acid and vinylsulphonic acid, vinylidene-1,1-diphosphonic acid and acrylic acid, vinylidene-1,1-diphosphonic acid and methacrylic acid, vinylidene-1,1-diphosphonic acid and acrylamide, vinylidene-1,1-diphosphonic acid, vinylsulphonic acid and acrylic acid, vinylidene-1,1-diphosphonic acid, vinylsulphonic acid and methacrylic acid, and vinylidene-1,1-diphosphonic acid, vinylsulphonic acid and acrylamide.

36. A coating according to claim 30, wherein said phosphonic acid polymer is poly(vinyl phosphonic acid-co-acrylic acid).

37. A coating according to any one of claims 30 to 36, wherein the molecular weight of the phosphonic acid polymer is in the range of around 100 g/mol to 500,000 g/mol.

38. A medical implant provided with a coating comprising a phosphonic acid polymer.

39. An implant according to claim 38, wherein the coating is in accordance with any one of claims 31 to 37.

40. A biocompatible fibre comprising a at least one type of phosphonic acid polymer.

41. A fibre according to claim 40, wherein said fibres are formed from a first type of phosphonic acid polymer.

42. A fibre according to claim 41, wherein said fibres are produced by electrospinning a solution of said first type of phosphonic acid polymer.

43. A fibre according to any one of claims 40 to 42, wherein fibres of the scaffold are provided with a coating containing a second type of phosphonic acid polymer.

44. A biocompatible polymer composition comprising polycaprolactone and a phosphonic acid polymer.

45. A composition according to claim 44, wherein the molecular weight of the phosphonic acid polymer is in the range of around 100 g/mol to 500,000 g/mol.

46. A composition according to claim 44 or 45, wherein said composition contains around 7 to 15 w/v% of the phosphonic acid polymer.

47. A method of culturing mammalian tissue cells, the method comprising culturing mammalian tissue cells on a fibrous tissue scaffold, fibres of the scaffold comprising a phosphonic acid polymer, and exposing said cells to a cell culture medium to facilitate adherence of said cells to the scaffold.

48. A method of repairing or regenerating mammalian tissue comprising culturing mammalian tissue cells on a fibrous tissue scaffold, fibres of the scaffold comprising a phosphonic acid polymer, exposing said cells to a cell culture medium to facilitate adherence of said cells to the scaffold, and placing the scaffold with cells adhered thereto adjacent to the mammalian tissue in need of repair or regeneration.

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Claims searched:	1-29 & 47	**Date of search:**	1 December 2009

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1-3, 5-10, 25-29 & 47-48	US2007/196663 A1 (SCHWARTZ) Whole document relevant, particularly paragraphs [0014], [0066] and [0120]-[0121].
X	1-3, 5-10, 25-29 & 47-48	WO2009/066879 A2 (KOREA INST CERAMIC ENG & TECH) Whole document relevant particularly page 4 line 21 - page 6 line 21.
X	1-3, 5-10, 25-29 & 47-48	WO2005/081699 A2 (UNIV FLORIDA) Whole document relevant, particularly claim 43.

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
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Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

A61L; C08G; C08L

The following online and other databases have been used in the preparation of this search report

EPODOC & WPI

International Classification:

Subclass	Subgroup	Valid From
A61L	0027/14	01/01/2006

Application No:	GB0914200.1	**Examiner:**	Dr Matthew Hall
Claims searched:	40-46	**Date of search:**	23 March 2010

Patents Act 1977
Further Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	40-41 & 43	WO2005/081699 A2 (UNIV FLORIDA) Whole document relevant, particularly claims 40-43

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Further Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	30-39	US2008/057097 A1 (BENCO) Whole document relevant, particularly paragraph [0022] and claim 17
X	30-39	US6645644 B1 (SCHWARTZ) Whole document relevant, particularly column 8 line 66 - column 9 line 8 and column 9 line 58 - column 10 line 2.
X	30-39	US2004/023048 A1 (SCHWARTZ) Whole document relevant, particularly paragraphs [0052] and [0069].
X	30-39	US6248811 B1 (OTTERSBACH) Whole document relevant, particularly claim 1.
X	30-31 & 38-39	WO2008/074154 A1 (UNIV BRITISH COLUMBIA) Whole document relevant, particularly paragraphs [0016]-[0019] and [0037].

Categories:

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