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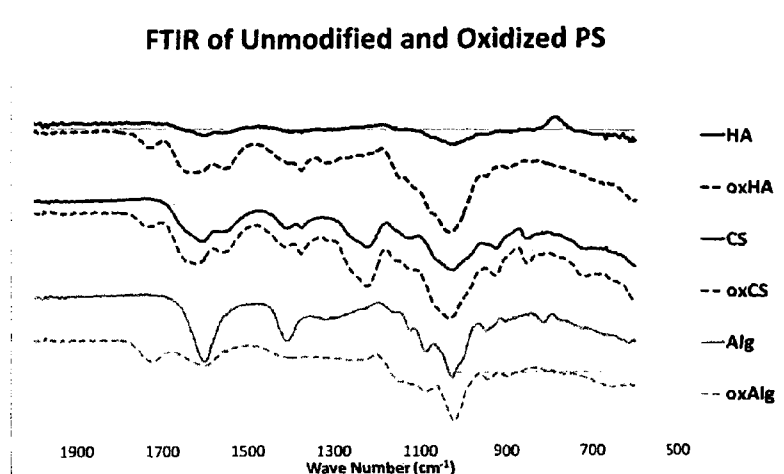
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Fig.5

FTIR of Unmodified and Oxidized PS



(57) Abstract: A method of cartilage repair, comprising the addition to a surface of cartilage damage of a crosslinkable scaffold material, the scaffold material comprising at least one cytocompatible polymer and at least one of minced tissue and cells, the crosslinking being provided by spontaneous reaction of complementary reactive groups of two types, at least one of these types being present on at least one of the cytocompatible polymer, minced tissue, cells and cartilage surface. The method offers a quick, easy and effective method of cartilage repair.

PROCESS OF CARTILAGE REPAIR

This disclosure relates to methods of activation of the surfaces of biological materials to enable their participation in cross-linking reactions, the result of which are biologically-engineered scaffolds for use in, for example, tissue repair and regeneration, especially that of cartilage.

In the repair of cartilage, there has been used clinically a number of basic cartilage lesion repair strategies. These, with their attendant disadvantages, are

- 10 • Marrow stimulating techniques, such as microfracture. This is a simple, common technique but one which has an up to 80% long term failure rate and can cause complications in the bone such as edema and cyst formation.
- Autologous chondrocyte implantation (ACI) or matrix-induced autologous chondrocyte implantation (MACI). These are regarded as the gold standard, but
15 several studies show that the outcome of ACI/MACI is no different than microfracture. ACI/MACI are expensive, 2 step procedures which rely on in vitro expansion of autologous cells, a process during which the cell lose their phenotype. The cells and matrix are held in place with a membrane and/or fibrin glue.
- Mosaicplasty. Autologous osteochondral discs are transplanted from non-weight-
20 bearing sites into the lesion. There is donor site morbidity and no healing of the spaces between the transplants.
- Particulated cartilage fragments of living tissue. Examples include cartilage allografts such as DeNovo NT and cartilage autograft implant system (CAIS).
25 There is limited tissue availability from juvenile cartilage donors in the first example and donor site morbidity in the second example. Both require fibrin glue to stabilize cartilage pieces to the defect.

Recently, decellularized tissues, that is, tissue in which the cells have been killed and their remnants removed, have attracted interest as scaffold material alternatives to simpler
30 approaches where the scaffold is composed of a single material (Hoshiba T, Lu H, Kawazoe N, Chen G. "Decellularized matrices for tissue engineering". Expert Opinion on Biological Therapy. 2010;10:1717-28). Tissue decellularization results in a scaffold of

extracellular matrix ideally suited for regenerating injured or diseased tissue since it retains the high resolution architecture and biological cues necessary for recapitulation of function. Decellularization has been applied to whole organs like the bladder with a fixed geometry (Horst M, Madduri S, Gobet R, Sulser T, Milleret V, Hall H, et al. "Engineering
5 functional bladder tissues". Journal of Tissue Engineering and Regenerative Medicine. 2012).

Devitalized/decellularized cartilage has not found widespread clinical use. Studies using both whole cartilage and covalent crosslinking of cartilage slurries have been attempted,
10 but these scaffolds cannot be attached to the native tissue surface except with glueing or suturing, and the latter involves heavy, toxic crosslinking and freeze-drying and is therefore not compatible with an in situ approach.

It has now been found that is it possible to avoid these problems and to provide a scaffold material that is easily created *in situ*, where it is desired. There is therefore provided a
15 method of cartilage repair, comprising the addition to a surface of cartilage damage of a crosslinkable scaffold material, the scaffold material comprising at least one cytocompatible polymer and at least one of minced tissue and cells, the crosslinking being provided by spontaneous reaction of complementary reactive groups of two types, at least one of these types being present on at least one of the polymer, minced tissue, cells and
20 cartilage surface.

The cytocompatible polymers (hereinafter referred to as "the polymers") for use in this method may be any suitable polymers with the necessary cytocompatibility, that is, their presence is not harmful to cells. They may be natural (biopolymers) or synthetic materials,
25 or combinations of these. The necessary complementary reactive groups may be already present on the polymers, or the polymers may be modified to include such groups. This is within the skill of the art in every case.

Typical non-limiting examples of natural polymers include alginate, alginate sulfate,
30 chondroitin sulfate, dermatin sulfate, hyaluronic acid, cellulose, dextran, poly-l-lysine, chitosan, gelatin, silk and collagen.

Typical non-limiting examples of synthetic polymers include polymers, or polymers derived from, poly ethylene glycol, poly propylene glycol, polaxomers, polyoxazolines, polyethylenimine, poly vinyl alcohol, poly vinyl acetate, poly methyl vinyl ether-co-maleic anhydride, poly lactide, poly N-isopropylacrylamide, poly glycolic acid, poly
5 methacrylate, poly acrylamide, poly acrylic acid, polyallylamine.

By “at least one” of the groups being present on at least one of the polymer, minced tissue, cells and cartilage surface, is meant that the complementary reactive groups may be present on all or any of these entities. For example,

- 10 (a) Both types present on different components, e.g. one on cartilage surface, the other on at least one of the polymer, minced tissue and individual cells;
- (b) Both types present on a single entity, for example, on the minced tissue, courtesy of, for example, oxidation and the amine groups naturally present.
- (c) Any combination of these possibilities.

15 Particular embodiments are further described hereinunder.

The size of the minced tissue to be used may be any suitable size, but in a particular embodiment, it is from 5 microns – 1 cm.

- 20 The minced tissue for use in the method may be any suitable soft tissue, but it is advantageously tissue of a similar or identical nature to that of the cartilage. Exemplary and non-limiting examples of suitable tissue include articular cartilage, nucleus pulposus, meniscus, trachea, nasal cartilage, rib cartilage, ear cartilage, synovial fluid, vitreous humor, brain, spinal cord, muscle, connective tissues, and liver. A particular example is
25 tissue with high carbohydrate content, which can be oxidized, particular examples being any type of cartilage, nucleus pulposus and meniscus. The tissue may be minced by any suitable method, exemplary and non-limiting methods including homogenizing, cutting, chopping, crushing, slicing and processing with a microtome.

- The tissue may be subject to decellularization to remove epitopes which can cause acute
30 inflammatory responses and pathogens including HIV. This may be done, for example, by using detergents, hydrogen peroxide, sodium hydroxide and enzymes, RNase and DNase.

The use of the term “cells” in this description encompasses not only individual cells, but also spheroids, pellets and microtissues, which are well known to and commonly used by the art. The cells for use in the method are advantageously cells of a similar type as those
5 present on the cartilage tissue. Typical non-limiting examples of suitable cell types include primary autologous chondrocytes, primary allogenic chondrocytes, chondroprogenitor cells, chondroblasts, mesenchymal stem cells, induced pluripotent stem cells and adipose-derived stem cells.

10 As hereinabove described, the crosslinking is provided by means of complementary reactive groups of two types, which are present on at least one of the minced tissue, the cells, the polymer and the cartilage surface, and which react spontaneously on being brought into contact. In a particular embodiment, the cartilage surface comprises reactive groups and the complementary reactive groups are present on at least one of the other
15 components. However, it is not essential that the cartilage surface have such groups, and in some cases, it may be free of such groups, all reaction being provided by the biopolymer, cells and minced tissue. In a further embodiment, minced tissue and cartilage surface may be oxidized to provide aldehyde groups and the linking provided by an amino-containing polymer

20

Many such complementary types of reactive groups are known, and any of the types may be selected, provided that (a) they can be provided in the necessary places, and (b) they do not adversely affect the desired repair. The skilled person will readily be able to select appropriate complementary reactive groups. For example, the skilled person would not
25 select glutaraldehyde, a well-known crosslinking agent for materials such as gelatin, because this would kill any cells in the vicinity.

Non-limiting examples of suitable complementary groups include those that result in Michael addition, disulfide bond formation, catechol-initiator polymerization, and enzyme-mediated crosslinking.

30 In a particular embodiment, the complementary groups are aldehydes and/or ketones and amines. This is the well-known Schiff base reaction, best known as one of the standard

tests for the presence of aldehydes, and Schiff bases prepared by this reaction are well known in fields of chemistry as diverse as perfumery, dyestuffs and liquid crystals. However, it has never been seen as a means of crosslinking tissue to form a scaffold material. Further discussion will refer exclusively to this reaction, although it is
5 emphasized that, as previously mentioned, other complementary groups are possible.

The necessary reactive groups may be there naturally, for example, the amine functionality of amino acids. This may also be true of aldehyde or ketone functionality, but it may also be needed to be provided by surface modification. For example, a tissue may be modified
10 by oxidation to provide the necessary groups or these groups attached with chemical linker molecules. Another possibility is to use a polymer that already contains the necessary groups.

In the specific example of the Schiff base reaction, aldehyde reactive groups may be
15 formed in minced tissue by chemical oxidation with reagents such as sodium periodate, sodium (meta) periodate, hydrogen peroxide, and horse radish peroxidase with hydrogen peroxide. Alternatively, physical and physical-chemical methods may be employed, for example, treatment by low temperature plasma and UV exposure, optionally in combination with hydrogen peroxide. Further methods include the formation of aldehyde
20 groups by incubation of tissue with light-absorbing dyes such as methylene blue, riboflavin, benegal rose and eosin Y in conjunction with light at wavelength from 200-1000 nm, particularly light in the UV range.

In the specific example of the Michael addition, this may be achieved, for example, by the reduction of disulfide bonds in tissue and tissue surfaces with tris(2-carboxyethyl)-
25 phosphine TCEP to introduce free sulfhydryl groups. This can also be achieved by the coupling of polymers with dithiobispropionic hydrazide (DTPH) with EDC followed by reduction to generate the free thiol, the conversion of amines to free thiols by 2-iminothiolane (Traut's Reagent), thiolation of proteins by N-succinimidyl S-acetylthioacetate (SATA) and the conversion of oxidized glycosaminoglycans to free thiols by 2-
30 acetamido-4-mercaptopbutyric acid hydrazide (AMBH): The free sulfhydryls can participate in scaffold formation and adhesion via reaction with Michael-type acceptors

including, but not limited to, acrylate esters, acrylonitrile, acrylamides, maleimides, alkyl methacrylates, cyanoacrylates and vinyl sulfones.

In the specific example of enzymatic crosslinking, crosslinking is effected by an enzyme. This method relies on the presence of functional groups, which are present already on the
5 minced tissue and tissue surfaces or which can be generated thereon. Typical non-limiting examples include horseradish peroxidase + hydrogen peroxide which catalyze the formation of covalent linkages between hydroxyphenols and transglutaminase which catalyzes the covalent bond between a free amine and carboxamide group of glutamine containing materials.

10 Many polymers, notably biopolymers, contain amino groups in their chemical structure naturally. Cartilage surfaces can be incubated in/under a solution of a polymer containing amino groups, which adheres to the surface via electrostatic interactions and physisorption. Similarly, resuspending cells in a solution of polymer results in deposition of the polymer onto the cell surface and 'coating' the cells with the polymer containing necessary amino
15 groups. This also happens spontaneously via electrostatic interactions or physisorption.

Carbohydrates such as alginate and hyaluronic acid can be oxidized by incubation with sodium periodate, which cleaves their vicinal hydroxyl groups, resulting in reactive aldehydes. Other reagents that can be used for chemical oxidation include sodium (meta)
20 periodate, hydrogen peroxide, and horse radish peroxidase. Carbohydrates present in the surfaces of minced cartilage particles can be oxidized chemically as above (sodium periodate, hydrogen peroxide, etc.).

In terms of the reactive groups, it is preferred to have a stoichiometry of as close to 1:1 as
25 possible. However, this is not narrowly critical, and a variation of up to 20% is tolerable. In the case of the aldehyde-amine reaction, it is preferred to have an excess of amines, as this is more tolerated by the cells. This can be assured, for example, by suspending the cells in a suitable material, such as chitosan.

30 Either or both of minced tissue and cells may be used in the scaffold material, and this will depend on the nature of the use. For example, when the area to be filled is relatively small,

of the order of a few cm^2 , cells with polymer in the absence of minced tissue may be used. For larger areas, minced tissue, either alone or with cells, may be used. What to use in what circumstances may be readily determined in each case by simple, non-inventive experimentation.

5

The composition of the scaffold material may be varied across wide limits, depending on the nature of the materials and the end-use. When cells are present, they are typically used at concentrations of 3×10^6 cells/mL - 50×10^6 cells/ml. When minced tissue is present, it is typically present at a weight proportion of from 10-40% tissue (to approximate the natural
10 content of cartilage. The polymer is typically present in a weight proportion of from 0.5-10%. The balance of the composition is mainly water.

In addition to the major components hereinabove described, the crosslinkable material may include other materials, present to confer particular properties on the material. One
15 particular example is lubricating proteins, useful in the applications of layers as described further hereinunder. Other examples include growth factors, cytokines, drugs, biologics, siRNA, DNA, polyphenols into the polymeric solutions, which could augment regeneration of the tissues.

20 In a particular embodiment, a patient's injured tissue is treated in the same way as the minced tissue/cells in order to cross-link the scaffold material directly to the injury site.

The crosslinkable scaffold material in its ready-to-use form is a readily mouldable solid that can easily be inserted into a damaged area of cartilage. Powders of the molecules and lyophilized minced cartilage can be stored and combined and rehydrated just prior to use.
25 In use, it is applied to the surface of a damaged cartilage which has been prepared with the necessary crosslinkable groups as hereinabove described, for example, by oxidation. The material can also be press fit into the lesion to increase mechanical stability.

For best results, the overall dry weight of the components in the implant scaffolds should be adjusted to match the volume of the defect site to be filled. In addition, the degree of
30 surface modification of the minced tissue and/or linker molecules is adjusted to optimize the stoichiometric relations of the cross-linking reactions and to promote highest degree of

adhesion within the scaffold and adhesion between scaffold and defect site.

The gelation time may be tailored to a particular application by altering the ratios of the mixed components to one another.

In a further embodiment, the implant may be applied in layers, rather than in a single
5 homogeneous mass. The individual layers can be varied, so as to more precisely mimic the structure of actual tissue. For example, at the surface of cartilage, there are present lubricating proteins. These could be included as part of an initial layer, and then excluded from subsequent layers, where they would not naturally occur. One way of achieving this is to use a 3D printer. Thus, a layer of cells/tissue particles/polymers would be printed,
10 followed by sequential layers, with the same or different composition. In such a layered approach, the crosslinking mechanism would take place not only within individual layers, but also between adjacent layers, thus forming a completely adhered whole.

The result is an *in situ* cartilage repair that is quick, effective and long-lasting. Typical examples of the use to which the method of this disclosure may be put include:

15

- re-surfacing and filling cracks and defects in articular cartilage and meniscus;
- securing or re-bonding loose tissues, such as a cartilage flap, in a defect site;
- filling spaces between mosaicplasty cylinders and interfacing them with native tissue;
- used as a bioadhesive for mechanically stabilizing a separate scaffold (e.g. collagen
20 gels used in MACI procedures) or implant material to a defect site with minimal or no need for sutures.

This method of this disclosure is highlighted by the following advantages:

25

- Utilizes autologous, allogenic or xenogenic native tissue which already contains the complex array of tissue-specific extracellular matrix components in physiologically accurate proportions. Aggrecan for example is extremely expensive in the purified form (\$400 /mg), but is abundantly present in cartilage fragments.
- After mincing, tissue fragments can be reconstituted and molded into any desired geometry to fill a defect without compromising its high-resolution biochemical composition.

- Suture-free method to adhere a tissue replacement material to an injury site or site of degeneration
- Both the treatment of donor tissue and its application to a site for adhesion rely on reactions which are completed in a time which is relevant for clinical applications
- 5 and can be incorporated into a 1-step surgical procedure
- Injectable components and in-situ scaffold formation could be applied in a minimally-invasive arthroscopic procedure
- Activation of tissue surfaces is done with simple reactions with possible applications in a number of different tissue types
- 10 • Possibility to incorporate therapeutic factors within the scaffold including, but not limited to: pharmaceutical compounds, growth factors, peptides, proteins, carbohydrates, and gene therapy vectors. Additionally, homing molecules can be included that would induce host cell migration into the scaffold.
- Possibility to achieve zonal organization of tissue architecture by layering various
- 15 tissues/compositions using additive manufacturing techniques (e.g. bioprinting)
- Possibility to tune the tribology properties of the surface layers of the scaffold through conjugation of molecules such a lubricin and superficial zone protein
- Possibility to include compaction step during construct formation to tune its mechanical properties

20

The disclosure is further described with reference to the following figures and non-limiting examples, which depict particular embodiments.

Figure 1 is a photograph of an intact tissue engineered construct composed of minced
 25 oxidized cartilage particles, oxidized chondroitin sulfate (oxCS), and succinylated chitosan (S-Ch). Construct was fabricated using a 24-well plate as a mold.

Figure 2 is a bar graph illustrating the mechanical properties of constructs composed of different ratios of oxidized minced cartilage (oxAC) and S-Ch with or without oxCS.

30

Figure 3 a) - d) shows a series of fluorescence microscopy images of cartilage discs on which cell adhesion experiments were performed. For visualization, cells were

fluorescently labeled with CellTracker™ Green CMFDA (5-Chloromethylfluorescein Diacetate, Life Technologies). The cartilage surface areas were too large to fit in a single field of view so images from neighboring field of views were taken, and the individual images were subsequently ‘stitched’ together using the microscope’s software (ZEN 2011, Zeiss) to give these pictures of the entire cartilage disc surface.

Figure 4(a) and (b) are bar graphs showing the adhesion performance of cells in Example 2.

Figure 5 is a Fourier transform infrared spectrograph. The individual traces correspond to the individual descriptive lettering beside them.

10

Figures 6 a) – d) are photographs of spherical microtissues comprised of 250 thousand cells each. In “a”, the sphere of cells was formed by centrifuging the cells in a conical tube and waiting 24 hours for the cells to form a sphere. The rectangles are scale bars that represent a distance of 500 micrometers in the image. In 6 b), c) and d), the microtissues were formed using oxPS and succinylated chitosan.

Figure 7 is a bar graph of gene expression results obtained by real time quantitative polymerase chain reaction (RT-qPCR), using collagen 2 as a marker of chondrogenic differentiation

Figure 8 is a bar graph of gene expression results obtained by real time quantitative polymerase chain reaction (RT-qPCR), using Aggrecan.

Figure 9 is a series of photographs covering the repair of a repair made in a bovine knee joint

Figure 10 is a micrograph of a scaffold made by the crosslinking of minced cartilage pieces using the Schiff base reaction.

Example 1: Oxidation of minced cartilage particles and crosslinking with n-succinyl chitosan

Glycosaminoglycans present on the surfaces of minced cartilage particles were oxidized with 5 minute incubation in a solution of sodium periodate (10% w/v in

dH₂O). Aldehyde presence was confirmed by incubation with Schiff reagent (Pararosaniline, 1% and sodium metabisulfate, 4%, in hydrochloric acid, 0.25 mol/L) and monitoring a color change to pink.

- 5 To prove the cross-linking capability of activated tissue fragments, oxidized cartilage pieces were mixed at a ratio of 1:1 with N-succinyl-chitosan (S-Ch, 2% w/v), a water-soluble chitosan derivative. Gelation occurred in about 30 seconds yielding a robust scaffold that could be comfortably manipulated with forceps (Fig. 1). The mechanical properties of this gel were investigated using a texture analyzer (Fig. 2).

10

To improve the compressive modulus of the construct, a structured model for assembly was used. A layer of oxidized cartilage particles was sandwiched between two layers of S-Ch and the entire construct was compressed to push out excess water, a byproduct generated during the Schiff linkage formation. Furthermore, oxidized
15 chondroitin sulfate was also added into the “sandwich” in the same layer as the oxAC in order to assure all amino groups available would participate in the Schiff linkage and improve construct stability.

Fig. 2 illustrates the ability to change the mechanical properties to suit the particular
20 use desired.

Example 2: Oxidized alginate as template for cell adhesion on cartilage surfaces

Droplets of 1 µL of oxAlginate were pipetted in a circle around the perimeter of
25 cartilage plugs (Ø = 8mm), and fluorescently-labelled cells in S-Chi were seeded on to the surface. As a comparison, identical plugs were prepared, except that the oxAlginate was replaced by a ring of saline solution (the current clinical standard procedure). The entire cartilage plugs were washed by dipping into PBS after 10 minutes and cells adhered only in locations on the surface where the oxAlginate was
30 initially.

The results are shown in Figures 3c (saline) and 3d (oxAlginate). It can be seen that very few cells remain adhered to the saline samples, whereas substantial numbers remain adhered to the oxAlginate.

5

Example 3 Oxidizing cartilage tissue surfaces to control cell adhesion

Cartilage plugs were prepared and the glycosaminoglycans thereon were directly
10 oxidized by brief incubation with 100 mg/mL sodium periodate. After 5 minutes of oxidation, cartilage plugs were washed thoroughly in PBS and seeded with cells resuspended in S-Chi.

As a comparison, similar plugs were prepared and given the same treatment, but not oxidized. The results are shown in Figures 3a (not oxidized) and 3b (oxidised)

15

It can be seen that cell adhesion in was significantly higher on the oxidized surfaces (Fig. 3b) than on unoxidized saline control (Fig. 3a). The cells adhere only to the prepared surface. This shows that more cells can be brought to and adhered to the exact location where they are needed.

20

The number of cells adhered to a cartilage surface was shown to depend on both the time of oxidation (Fig. 4a) and the concentration of sodium periodate used (Fig. 4b)

Polysaccharide oxidation (oxPS) was confirmed by Fourier transform infrared
25 spectroscopy (FTIR) (Fig. 5) and the appearance of a distinct peak at 1730 cm^{-1} representative of the aldehyde bond created in the reaction.

Example 4 Formation of mimetic microtissues by crosslinking cells

30 To form microtissues using the Schiff linkage technique, human mesenchymal stem cells (hMSCS, Lonza Group Ltd, Basel, Switzerland) between p.6-p.9 were suspended in a solution of 5 mg/mL sChi at a cell density of 20×10^6 cells/mL. Drops of 5 μL

each of the corresponding oxPS molecules were prepared on plastic ring structures to confine the drop and 10 μ L of the cells + S-Chi were pipetted into individual droplets of oxPS. The substrate was turned upside down and the reaction was carried out in an incubator at 37°C for 10 minutes. Microtissues were transferred to agarose coated-
5 wells of a 96-well plate with fine-tipped forceps and cultured in chondrogenic media (Fig. 6 b-d) oxCS, oxHA, and oxAlg resp.). In parallel, hMSCs were centrifuged to form micromass pellets (200k cells/each) for comparison and transferred to the same 96-well plate (Fig. 6a). Microtissues formed via Schiff-base crosslinking using S-Chi and oxAlg demonstrated better chondrogenic induction than centrifuged pellets as
10 indicated by gene expression of type II collagen (Fig. 7) and aggrecan (Fig. 8).

These specific qPCR data were taken after 21 days of stem cell culture using the two different systems and show that microtissues crosslinked with oxidized alginate (oxAlg) induced 21,000x higher expression of collagen 2 and 5,000x higher
15 expression of aggrecan than cells cultured by centrifugation.

The data indicate that crosslinked microtissues provide an immensely beneficial environment for differentiating stem cells into cartilage cells, versus the traditional technique of the art (i.e. centrifugation).

20

Example 5 Formation of scaffolds based on minced cartilage to fill cartilage defects:

Articular cartilage from a 6 month bovine knee was minced with a razor blade to pieces
25 approximately 1 mm x 1mm x 1mm. The cut pieces were then oxidized in 123.4 mg sodium periodate per 1 mL minced tissue (volume after centrifugation) in 20mL ddH₂O. After overnight oxidation, the tissue particles were lyophilized to reveal a separation of the sample into hard pieces (primarily collagen) and a softer fluff (glycosaminoglycans). 50mg of collagen pieces, 50 mg of the glycoaminoglycan
30 component were combined with 200 μ L of 2% succinylated chitosan (60% amino substitution). The slurry was combined with a flat spatula and used to fill a previously made defect in a cadaver bovine knee which had been just treated with 1% sodium

periodate for 30 s. The slurry was press fit for 3 minutes to allow for crosslinking. The entire process is shown in Figures 9 A-D, in which

9A shows the 8mm defect which has been surface-oxidised, as described above;

9B shows the ground, oxidised cartilage particles mixed with succinylated-chitosan;

5 9C shows the filling of the defect with the mixture; and

9D shows the filled defect.

The result was the complete filling of the defect with a mechanically stable, biocompatible material which is covalently crosslinked to the tissue surface.

10 The micrograph of Figure 10 shows a scaffold prepared from the minced cartilage and succinylated chitosan press-fitted into a cylindrical hole and then removed.

CLAIMS

1. A method of cartilage repair, comprising the addition to a surface of cartilage damage of a crosslinkable scaffold material, the scaffold material comprising at least one cytocompatible polymer and at least one of minced tissue and cells, the crosslinking being provided by spontaneous reaction of complementary reactive groups of two types, at least one of these types being present on at least one of the polymer, minced tissue, cells and cartilage surface.
2. A method according to claim 1, in which the cytocompatible polymer is a natural polymer.
3. A method according to claim 2, in which the cytocompatible polymer is selected from the group consisting of alginate, alginate sulfate, chondroitin sulfate, dermatin sulfate, hyaluronic acid, cellulose, dextran, poly-L-lysine, chitosan, gelatin, silk and collagen.
4. A method according to claim 1, in which the cytocompatible polymer is a synthetic polymer.
5. A method according to claim 4, in which the polymer is selected from the group consisting of polymers, or polymers derived from, poly ethylene glycol, poly propylene glycol, polaxomers, polyoxazolines, polyethylenimine, poly vinyl alcohol, poly vinyl acetate, poly methyl vinyl ether-co-maleic anhydride, poly lactide, poly N-isopropylacrylamide, poly glycolic acid, poly methylmethacrylate, poly acrylamide, poly acrylic acid, polyallylamine.
6. A method according to claim 1, in which the cartilage surface comprises one type of complementary reactive group.
7. The method of claim 1, in which the minced tissue is in the range of from 5 microns - 1 cm.

8. The method of claim 1, in which the minced tissue is derived from tissue selected from the group consisting of articular cartilage, nucleus pulposus, meniscus, trachea, nasal cartilage, rib cartilage, ear cartilage, synovial fluid, vitreous humor, brain, spinal cord, muscle, connective tissues, and liver.
- 5 9. The method of claim 1, in which the minced tissue has a high carbohydrate content.
10. The method of claim 1, in which the cells are selected from the group consisting of primary autologous chondrocytes, primary allogenic chondrocytes, chondroprogenitor cells, chondroblasts, mesenchymal stem cells, induced pluripotent stem cells and adipose-derived stem cells.
- 10 11. The method of claim 1, in which the biopolymer is selected from the group consisting of oxidized alginate, oxidized hyaluronic acid, oxidized chondroitin sulfate, oxidized cellulose, succinylated chitosan, gelatin, silk and collagen.
12. The method of claim 1, in which the complementary reactive groups are those
15 selected from groups which participate in Michael addition, disulfide bond formation, catechol-initiator polymerization and enzyme-mediated crosslinking.
13. The method of claim 1 in which the complementary reactive groups are aldehyde/ketone and amine.
- 20 14. The method of claim 13, in which the aldehyde/ketone groups are provided on the minced tissue and/or cartilage surface by oxidation.
15. The method of claim 1 where the biopolymers and/or minced cartilage can act as
25 reservoirs for growth factors and other mitogenic factors to promote healing and regeneration.
16. A method of claim 1, in which the crosslinked scaffolding material is used to re-surface and re-fill cracks and defects in articular cartilage and meniscus.
17. A method of claim 1, in which the crosslinked scaffolding material is used to secure

or re-bond loose tissue in a defect site.

18. A method of claim 1, in which the crosslinked scaffolding material is used to fill spaces between mosaicplasty cylinders and interface them with native tissue.
19. A method of claim 1, in which the scaffold material comprises additional
5 components.
20. A method according to claim 19, in which the additional components are selected from lubricating proteins, growth factors, cytokines, drugs, biologics, siRNA, DNA and polyphenols
21. A method according to claim 21, in which the additional component is lubricating
10 protein and the scaffolding material is applied in layers of differing compositions, that layer near the cartilage surface comprising lubricating proteins, and subsequent layers comprising fewer and finally no such proteins.

Fig.1



Fig. 2

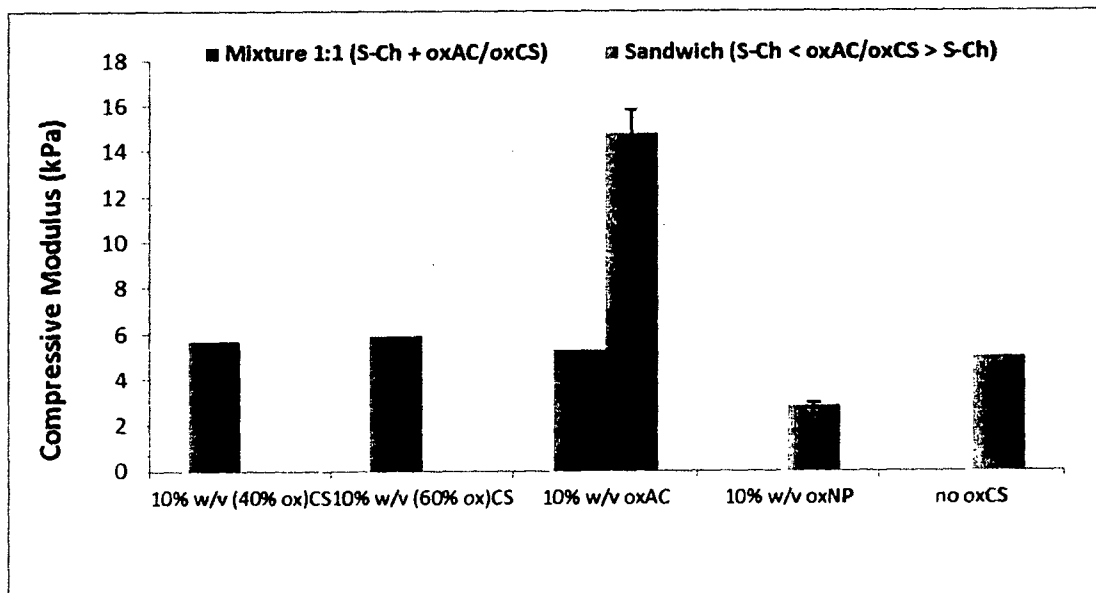


Fig.3

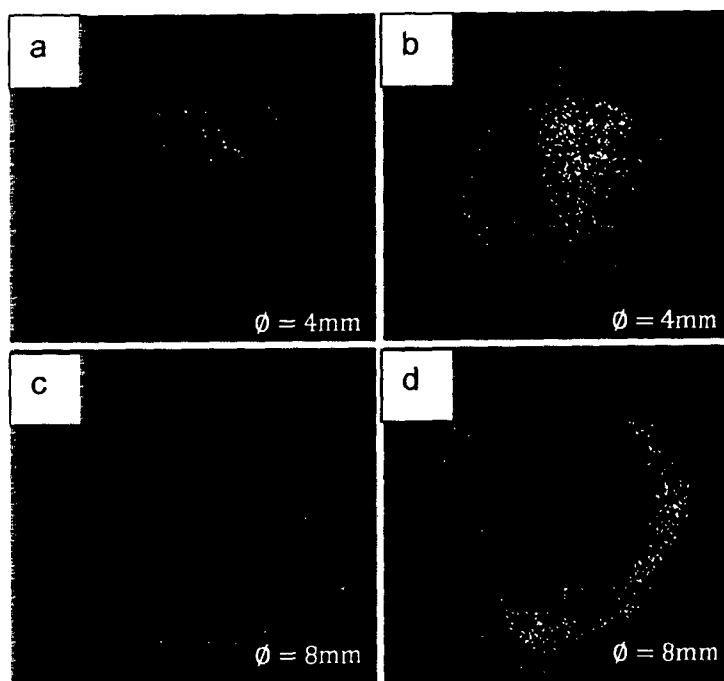
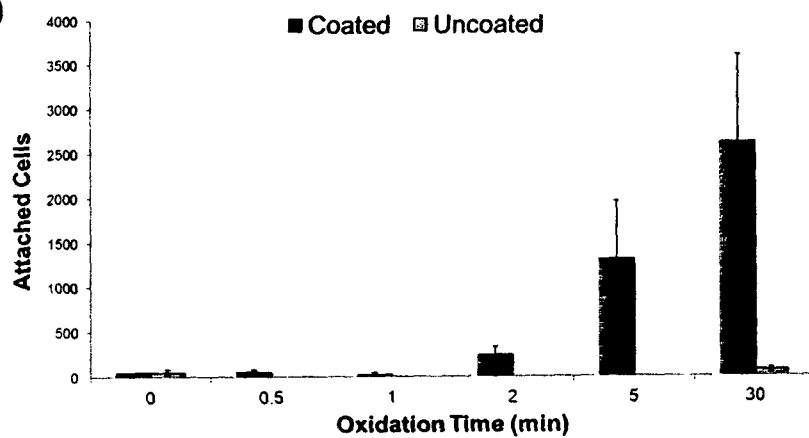


Fig.4 (a)



(b)

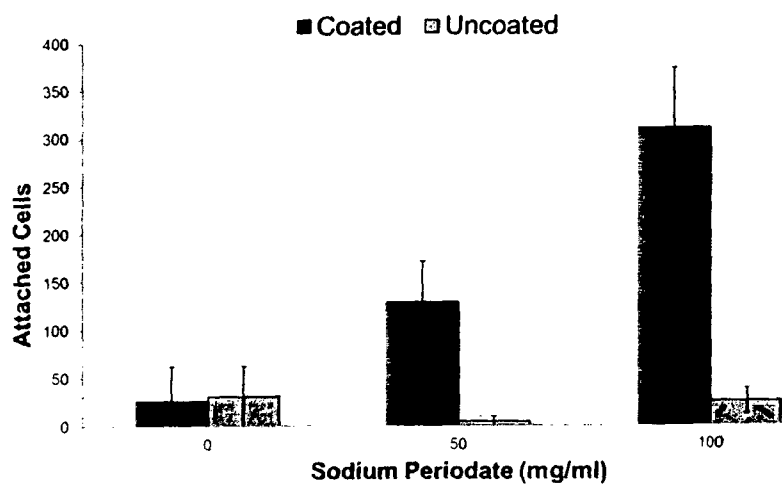


Fig.5

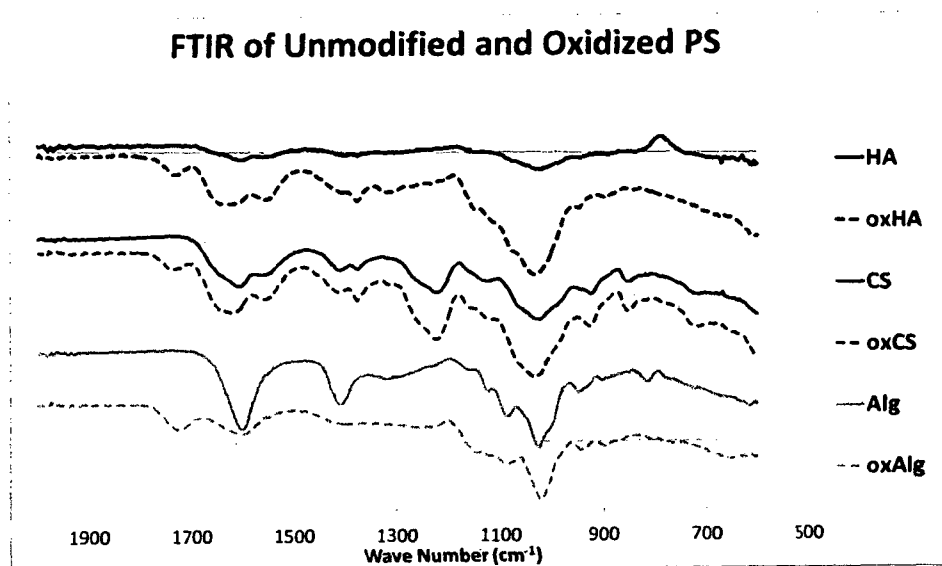
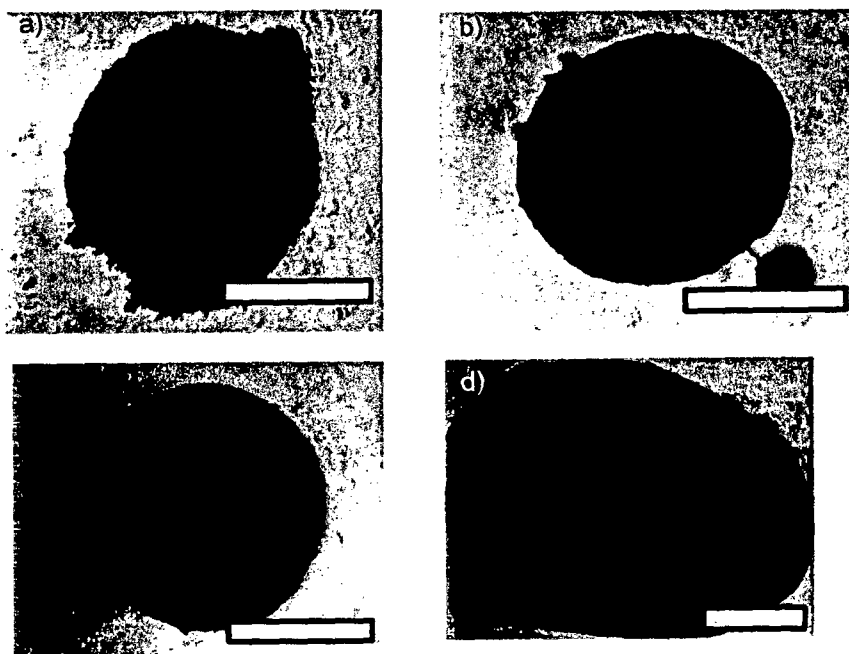


Fig.6



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Fig.7

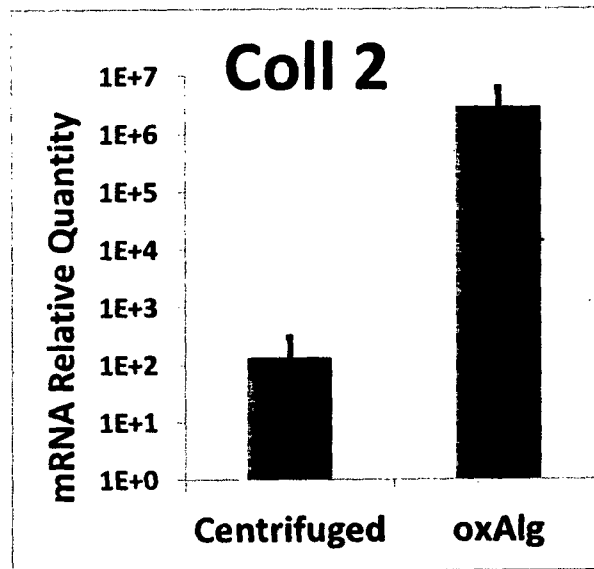


Fig.8

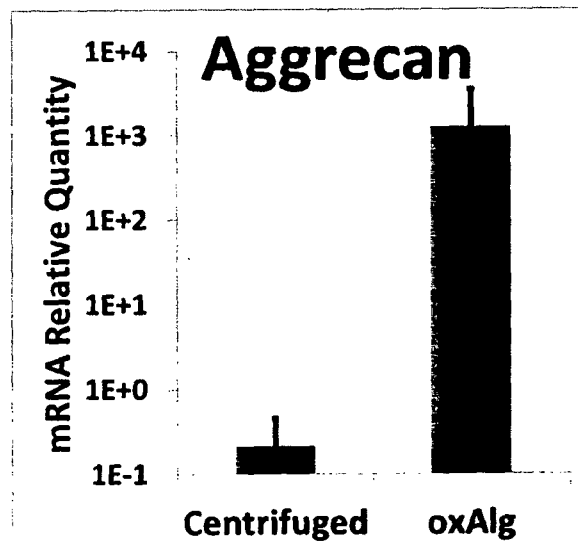
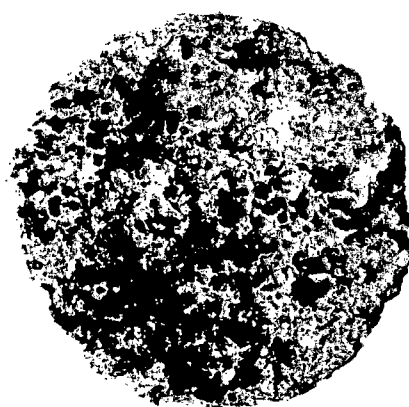


Fig. 9



Fig.10



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/001931

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	A61L27/16	A61L27/18 A61L27/20 A61L27/22 A61L27/24
ADD.	A61L27/36	
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		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
EPO-Internal, COMPENDEX, EMBASE, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 506 790 A1 (DEPUY MITEK INC [US]) 16 February 2005 (2005-02-16) paragraph [0037] - paragraph [0038] paragraph [0062] - paragraph [0063] paragraph [0068] - paragraph [0072] paragraph [0076] paragraph [0088] ----- -/-	1-5, 7-11, 15-17, 19,20
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
15 August 2013		22/08/2013
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Zalfen, Alina

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/001931

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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International application No

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