Effect of fibre Cross-linking on Collagen-fibre reinforced Collagen-chondroitin-6-sulphate materials for regenerating load-bearing soft tissues

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Abstract

Porous collagen-glycosaminoglycan structures are bioactive and exhibit a pore architecture favourable for both cellular infiltration and attachment; however, their inferior mechanical properties limit use, particularly in load bearing situations. Reinforcement with collagen fibres may be a feasible route for enhancing the mechanical characteristics of these materials, providing potential for composites used for the repair and regeneration of soft tissue such as tendon, ligaments and cartilage. Therefore, this study investigates the reinforcement of collagen - chondroitin-6-sulphate (C6S) porous structures with bundles of extruded, reconstituted type I collagen fibres.

Fibre bundles were produced through extrusion and then, where applicable, cross-linked using a solution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) / N-hydroxysuccinimide (NHS). Fibres were then submerged in the collagen–C6S matrix slurry before being lyophilised. A second EDC and NHS cross-linking process was then applied to the composite material before a secondary lyophilisation cycle.

Where bundles had been previously cross-linked, composites withstood a load of approximately 60 N before failure, the reinforcing fibres remained dense and a favourable matrix pore structure resulted, with good interaction between fibre and matrix. Fibres that had not been cross-linked before lyophilization showed significant internal porosity and a channel existed between them and the matrix. Mechanical properties were significantly reduced, but the additional porosity could prove favourable for cell migration and has potential for directing aligned tissue growth.

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Introduction

Extra-cellular matrix (ECM), the structural basis of soft tissues, is an elaborate macromolecular structure that defines the physical morphology of tissues and dominates the local environment surrounding cells[1]. Whilst the specific nature of the ECM will vary depending upon tissue type, collagen is always a major constituent, along with gel-like proteoglycans. In connective tissue such as ligaments and tendon, the tissue is often under complex mechanical loading having to transmit tensile loads, provide connective flexibility, permit body locomotion and enhance joint stability[2]. Soft tissues in more compressive environments such as cartilage, menisci and the tendon enthesis undergo continuous cyclic loading and also provide a physical support structure utilising type-I and type II collagen fibres. All connective tissue must also support cellular activity not least through the sequestration and interaction with numerous soluble regulators of cell behaviour[1].

Unfortunately damage to connective tissues is prevalent and often associated with significant morbidity. It has been estimated that approximately 80,000 ACL repairs are carried out each year in the US[3] and in 2002, 232,000 Achilles tendon injuries occurred in the US; of these at least 66,000 required hospital treatment[4]. In a 5 year study at an Edinburgh hospital, all outpatient and inpatient visits for soft tissue tendinous or ligamentous injury were investigated[5]. Meniscal injury of the knee was the most common with an incidence of 23 / 100,000 local population, whilst Achilles tendon rupture had an incidence of 11.3 / 100,000 and ACL rupture of 8.1 / 100,000. Although tendons and ligaments are able to heal naturally to a certain extent, pre-injury conditions are not restored as a result of scar tissue formation, which has inferior mechanical characteristics[2]. Cartilage repair can prove to be even more problematic as the lack of blood supply results in an absence of chondrogenic cells in the healing defect[6]. Large defects in particular require surgical intervention and whilst autologous (i.e. autologous chondrocyte implantation, bone-patellar-bone ACL reconstruction) and allogenic (ACL grafts etc) materials are applied with some success, the use of (bio)synthetic

materials as scaffolds designed for cellular infiltration and tissue recapitulation are also increasingly being applied in the field of soft tissue repair.

A promising route for tissue repair is via the implantation of collagen-based material that enables the infiltration and proliferation of the patient's own cells in the scaffold, leading to the production of new ECM and resulting in regeneration of native tissue characteristics. Freeze-dried porous collagen-glycoscaminoglycan (GAG) biomaterials have been applied for soft tissue regeneration including skin[7-9], nerve regeneration[10], meniscus[11] and cartilage repair[12-14]. They are highly bioactive and exhibit a pore structure favourable for both cellular infiltration and attachment *in vitro*[15, 16] and tissue regeneration *in vivo*[17]. However, they exhibit inferior mechanical properties, particularly for applications requiring strength in tension. Extruded collagen fibres, reconstituted from acid swollen gel type I collagen have been investigated particularly for the purposes of ligament and tendon repair[18]; but implant definition, ease of implantation and cellular attachment may be limited for a fibre-only material. A combination of a freeze-dried collagen based matrix with collagen fibre reinforcement has been previously considered in the production of bioartificial dermis[19] and may prove equally applicable in the repair of tissues such as ligament, tendon and cartilage.

When collagen is generated *in vivo*, cross-linking occurs enzymatically and covalent inter and intra-molecular bonds are formed that confer the desired mechanical characteristics and proteolytic resistance. Unfortunately however, these cross-links are not formed to the same extent when collagen fibres self-assemble in neutral pH in vitro[20]. There are a number of cross-linking approaches including chemical (glutaraldehyde, isocyanates or carbodiimide based)[18], physical (dehydrothermal treatment)[21] and enzymatic[22]. Selection of cross-linking approach has a significant effect on physical and biological characteristics.

Cross-linking of collagen based materials using zero-length linkers such as the carbodiimide 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) is favourable because the cross-linking occurs without incorporation of the cross-linking agent into the material. Where a glycosaminoglycan such as chondroitin-6-sulphate is also present, it is able to act as an additional cross-linking agent, with the EDC activating its CH_6SO_4 groups. Cross-linking is a 2-step process – in the first step an accessible carboxyl group is activated by the carbodiimide to form an intermediate 0-acylisourea (a carboxylic ester with an activated leaving group), this intermediate can then condense with an amino group of collagen to yield amides. Amide formation is thus obtained between the CH_6SO_4 groups of the chondroitin-6-sulphate and collagen or collagen and collagen[23]. This route however tends to result in a lower cross-linking density when compared with other chemical as well as physical cross-linking methods. Whilst other cross-linking routes particularly those using glutaraldehydes have been associated with a moderate inflammatory response and reduced bioactivity[24], EDC has been shown to have minimal effect on biocompatibility[25]. EDC cross-linked fibres have been observed to swell significantly more than those cross-linked by other routes[25] suggesting the presence of a lower crosslink density and more hydrophilic surface. Hydrophilic surfaces have been shown to enhance the biological activity of fibronectin, a key protein associated with cell attachment, proliferation and migration, and possibly a key factor in the bioactivity of EDC cross-linked collagen[26].

In this paper, lyophilised structures of collagen-chondroitin-6-sulphate combined with extruded collagen fibres have been investigated for production of a bioactive and biomechanical tissue engineering material. A carbodiimide based cross-linking method was applied and the effect of cross-linking of the fibre bundle prior to composite formation investigated.

Materials and Methods

Fibre extrusion

Fibres were extruded using a method based on the patent of Silver et al.[29] and an experimental set-up summarised in *Figure 1a*. Frozen acid swollen gel type I collagen from bovine dermis (Devro Medical, Moodiesburn, Scotland) was added to 2 mM HCl at a concentration of 6 mg mL^{-1} and refrigerated overnight before being blended and degassed. This collagen slurry was then drawn into two 30 mL syringes each with a three thread manifold attached (each thread of inner diameter 0.51 mm), allowing six collagen strands to be simultaneously extruded. The collagen was extruded at a controlled rate of 0.9ml/min (0.3ml/min through each thread) into a flowing bath of 20% w/v solution of polyethylene-glycol (PEG) (molecular weight 8000) in phosphate buffered saline solution, spending approximately five minutes in the bath. The six strands were collected together at the end of the extrusion bath using tweezers and wound onto a rotating spool in the form of a six ply fibre bundle as demonstrated in *Figure 1a*. Horizontal motion of the spool meant that a continuous length of collagen fibre was produced largely without overlap; a typical wound spool is imaged in *Figure 1b*. 15ml of the collagen slurry was used per fibre bundle. The collagen fibre bundle was left on the spool to dry overnight.

If cross-linking was carried out at this stage, the fibre bundles were immersed in a cross-linking solution of 25 mM EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) and 12.5 mM NHS (N-hydroxysuccinimide) in an 80/20, acetone / phosphate buffered saline (PBS) solution mixture for 2 hours. The fibre bundles then underwent a multi-stage washing process with PBS solution and deionised water. Three litres of cross-linking solution were used per 10 collagen bundles, resulting, according to the work of Olde-Damink *et al* in an excess of the two cross-linking agents (compared to the number of carboxylic acid groups present)[30].

Bundles where cross-linking was not applied at this stage underwent the multi-stage washing process to ensure full removal of the PEG. All samples were then dried overnight before being removed from the spools and stored. A typical dried and cross-linked fibre bundle after removal from the spool is shown in *Figure 1c*.

Composite formation

The composite material consisted of a collagen fibre bundle immersed in a collagen–chondroitin-6-sulphate matrix. Although fibre volume after freeze-drying was relatively low at approximately 6%, the fibres made up 75% of the dried mass of the final samples. The matrix slurry was produced through the blending of freeze-dried type I collagen from bovine dermis (Devro Medical) with sodium salt chondroitin-6-sulphate (BioIberica) to form a solution of concentration 0.92% collagen, 0.08% chondroitin-6-sulphate by weight, in 2mM HCl. After blending, the slurry was vacuum degassed and poured into a metallic plate (medical grade stainless steel) containing insets of dimension 60mm*10mm*5mm as the mould for the fibre reinforced material. 3ml of collagen slurry was used per sample, with a proportion of the slurry added, then the fibre bundle and then the remainder of the slurry. Fibre bundles were added whole, with fibre alignment parallel to the length of the samples. The fibre bundle length was approximately 10mm longer than the inset length, and ends of the fibre bundles (region of the bundle in contact with the spool rods) remained outside of the slurry during the freeze-drying process. The samples were initially frozen at -12˚C and dried under vacuum for 20 hours with a primary drying temperature of 0˚C and vacuum of 80 mTorr. Fibre bundle ends, above the surface of the matrix material were removed after the freeze-drying cycle.

After the first freeze-drying cycle, the composite material was cross-linked using the same solution of EDC and NHS described previously (33 ml of cross-linking solution per composite sample), to help increase the strength of the matrix material and provide a chemical interaction between matrix and the reinforcing bundles. Samples were then washed thoroughly before being placed back in the freeze dryer and the drying cycle repeated.

Matrix samples were also produced without fibre reinforcement for mechanical comparison, using the same freeze-drying profile and cross-linking method described above.

Characterisation

All composite samples were imaged using SEM (JEOL 820) to investigate pore morphology, fibre structure and interaction between fibre and matrix. To evaluate the fibre appearance after freeze-drying, the swelling behaviour of the collagen fibres was investigated using optical microscopy (GX Microscopes L2000B HTG) and measurements carried out using ImageJ software. Swelling was defined in terms of the % increase in diameter and significance determined using ANOVA followed by the two-tail student T-test with unequal variance at the 95% significance level (Microsoft Excel). The composite samples were tested under both tension and compression, after soaking in PBS solution for one hour, using an Instron 3343. For tensile testing, the rectangular cross-section samples (approximately 60mm*10mm*5mm as produced) were tightened within the grips with the fibre direction parallel to the tensile axis. A constant extension rate of 10 mm/minute was applied. Load was recorded using a 100 N load cell and tensile moduli at 3-5% strain calculated. For compression testing, samples of dimensions approximately 10mm*10mm*5mm were sectioned and a 2.5 N load cell used. Loading was carried out perpendicular to the fibre orientation, to mimic the load profile in natural soft tissue under compression. The samples

were pre-conditioned by applying 10 cycles between a compressive extension of 0.25 mm and 0.05 mm at a rate of 10mm /min to ensure all samples were in the same condition before testing was initiated. Samples were compressed at a rate of 10mm /min in an unconfined test with compressive moduli at 10-15% (low strain) and 55-60% (high strain) determined. Statistical analysis was carried out using ANOVA and Tukey's HSD.

Results

The photographs in *Figure 2* show that good replication of the mould shape was achieved when the fibre bundles were used both with and without prior cross-linking. Some degree of shrinkage was observed in all cases along with a ridge along the upper surface, likely to be a result of surface tension effect with the walls of the mould. Although slurry was added to the base of the mould before the first fibre bundle was placed, fibres were clearly evident at the lower surface of the samples and appeared to have a larger diameter in the instances where they did not undergo previous cross-linking (d).

As illustrated in *Figure 3*, the collagen-chondroitin-sulphate material exhibited a pore structure characteristic of freeze-dried samples with open and interconnected porosity and an average pore size estimated to be of the order of 100 μm. In the case of the fibres used with prior cross-linking (*Figure 3*(*a*) – (*c*)), dense fibres were clearly evident with little internal porosity or cracking; damage to the fibres as a result of the freeze-drying process appeared minimal. However, images (*d*) to (*f*) showed very significant internal porosity within the non-cross-linked fibres. A porous structure very similar to that observed in the matrix material was observed through the section of fibres. The absence of prior cross-linking did not appear to improve interaction between fibres and matrix and in fact larger channels were evident

around fibres that had not been cross-linked compared with those that had. No evidence of the 6 ply nature of the fibres was found in either cross-linked or non-cross-linked examples.

For porosity to form within the fibres, water from the matrix slurry needs to have penetrated the collagen fibres and then frozen and sublimed during the freeze-drying process. Optical microscopy was therefore carried out in order to investigate whether the internal fibre porosity could be correlated with a greater degree of water absorption in the case of the non-cross-linked fibres. The optical micrographs of *Figure 4 (a-f)* show the degree of swelling for sections of cross-linked and non-cross-linked fibre. Swelling in both cases was considerable and rapid, no significant variation is observed between the diameter after 10 minutes soaking and that after 1 hour soaking. When measured as a % of the original, the mean swelling of the non-cross-linked fibres (*Figure 4 (g)*) was higher after both 10 and 90 minutes soaking, but the variation between it and the swelling of the cross-linked fibre bundles was not statistically significant. Even in the case of cross-linked fibres a swelling in excess of 50% was observed. It is likely that swelling of both cross-linked and non-cross-linked fibres would actually have been more significant in the acidic freeze-drying conditions as a neutral pH has been shown previously to stabilise the collagen gel network[31]. It therefore appears that the absence of internal porosity within collagen fibres cross-linked before composite formation cannot be attributable to an absence of swelling. The strength and density of the chemical cross-links within the fibres must have been sufficient to hold the collagen fibrils together during freezing and sublimation of the solvent.

Mechanical Testing

Tensile Testing

Composite material containing fibre bundles that were not cross-linked before matrix addition showed a significant reduction in the tensile properties compared with those where prior cross-linking was applied (*Figure 5* and *Table 1*). The ultimate tensile strength was reduced by over 6-fold and the strain to failure as well as tensile modulus were also significantly reduced in the case of the non-cross-linked samples. With samples containing previously cross-linked fibre bundles, the tensile stress generally increased smoothly until close to the ultimate tensile strength, after which point failure of the reinforcing fibres occurred rapidly until final failure. Behaviour appeared broadly consistent with the 'j' shaped stress-strain curve of wet collagen fibres[32] associated with the progressive orientation of the fibres during straining. However with the non-cross-linked bundles failure of the individual fibres appeared to occur progressively with the load being taken up by the remaining fibres perhaps suggesting individual fibres were not equally tensioned within the bundle. The photographs of *Figure 6* were taken during the tensile test and whilst matrix material is evident around the tensioned fibres in (a) and (b), (samples including fibre bundles that had been previously cross-linked), in (c) the matrix material clearly failed early in the test after which all load was carried through the fibres. This supports the conclusion that there is better integration of the fibres into the collagen-C6S matrix for collagen fibres previously cross-linked which was suggested by SEM imaging. Both fibre bundles with and without prior cross-linking offer statistically significant advantage in terms of tensile properties over matrix only material as clearly demonstrated in *Table 1*. Increase in ultimate tensile strength is over 10 times in the case of the fibre bundle without prior cross-linking and over 100 times when the bundle is cross-linked before integration within the matrix slurry. Strain to failure and tensile modulus are also significantly increased by the presence of the fibre reinforcement.

Compression Testing

Whilst under tension, fibre addition was observed to impart significant mechanical advantage, the compressive behaviour (*Figure 7*) and mean compressive moduli in *Table 2* exhibited no significant variation between the composites using fibre bundles with or without previous cross-linking and the matrix-only material. Compressive moduli, even at 55-60% strain were less than 20 kPa, which is much lower than soft tissues such as cartilage, which has a compressive modulus of the order of 5 MPa[33]. Whilst the fibre loading appeared sufficient to provide reasonable tensile properties and to allow clear variation to be observed between the two bundle treatments, compressive properties were clearly dictated by the low stiffness matrix material and fibre loading would require significant increase if compressive properties were a key criteria.

Discussion

To the authors' knowledge, there is only one previously published study investigating the feasibility of reinforcing porous collagen-GAG structures with extruded collagen fibres[19]. Seo *et al* produced reinforcement through the creation of a relatively complex mesh of individual extruded collagen fibres overlaid by hand and then cross-linked using UV radiation. As in this current work, the fibre construct was immersed in the collagen-chondroitin-6-sulphate suspension before freeze-drying, cross-linked using EDC / NHS and freeze-dried for a second cycle. Tensile testing of these fibre reinforced constructs gave an average ultimate tensile strength of 1.5 ± 0.05 MPa, similar to the samples investigated here with the fibres with prior cross-linking. Whilst their samples had the advantage of isotropic properties, certainly in 2 dimensions (desirable for their application as artificial dermis), soft tissues such as tendon and ligaments are generally only tensioned along a single axis and as such anisotropic properties may be desirable. It is hypothesized that alignment within the

tissue engineering construct will result in the same alignment within the regenerated tissue, hence the simple uniaxial alignment of fibres implemented here.

It was initially hypothesised that if fibres were cross-linked before being combined with the matrix slurry, integration into the matrix would be limited due to the majority of the fibres' functional groups being used up during the first cross-linking stage, thereby resulting in in insufficient sites remaining for suitable reaction with the matrix material. In the situation where cross-linking had not already been carried out on the reinforcing fibres, it was logical to assume that cross-linking of the composite material should result in chemical linkages between the carboxyl groups of the collagen fibres and the carboxylic acid and CH_6SO_4 groups of the collagen-GAG matrix. However, the SEM images of *Figure 3* showed no improved integration between the two when the fibre bundles had not been previously cross-linked, yielding instead larger channels. When tension was applied, a much higher degree of integration between fibres and matrix was evident with fibres that had undergone previous cross-linking.

The initial hypothesis does not however take into account effects of the first freeze-drying cycle. Ice crystals are likely to nucleate around the fibre during the first freeze drying cycle, forming a channel between the fibre and surrounding matrix and, providing collapse did not occur during the cross-linking, any interaction between fibres and matrix would be minimal. Channels could have formed around the fibres without prior cross-linking as a result of an increased propensity for ice nucleation around the fibres or due to shrinkage of the fibres during the freezing process. No significant thermal conductivity variation is expected to occur as a result of cross-linking, so this is unlikely to explain the channel formation and shrinkage effects appear counterintuitive given the relative shrinkages of both fibre types.

Both the cross-linked and non-cross-linked fibres were observed to swell to a significant degree and given the porosity within the non-cross-linked fibres, and the complete absence of any porosity with those that had been previously cross-linked, if any shrinkage was to be expected it would be with those fibres that had been previously cross-linked. Surface variations of the fibres were not studied in this work and it may well be either a morphological or chemical surface effect that causes interaction between the matrix material and the previously freeze-dried fibres during the first freeze-drying stage. This is something that clearly requires further investigation; however given the very limited strength of the collagen-GAG matrix it is unlikely to have a significant effect on the overall mechanical properties of the composite.

The difference in the mechanical properties of composites reinforced using fibres with and without prior cross-linking is likely to be a result of the degree of cross-linking present within the collagen fibres. Where cross-linking only occurred after significant internal porosity had been generated as a result of the freeze-drying process, chemical bonding was limited by the absence of a dense packing of collagen fibrils. Whilst cross-linking of the fibre bundles prior to composite formation was associated with improved tensile behaviour, the internal porosity of the fibres freeze-dried without previous cross-linking may well offer some advantages biologically. Fibroblast migration has been shown to be elevated away from cell junctions in porous collagen structures[34] and as such long aligned channels can only be beneficial for cell migration and ultimately aligned tissue growth. Although integration between fibre and matrix is not of particular significance under tension where properties are fibre dominated or under compression, when composite materials are placed under shear, a common loading situation *in vivo,* bonding between matrix and fibre is of much greater importance.

Human tendons and ligaments exhibit ultimate tensile strengths in the range of 30-100 MPa[35], so the composites tested here require further development before they achieve the mechanical properties of the soft tissues they are intended to repair. There certainly exists the capability of enhancing the mechanical properties by increasing the fibre fraction significantly; however, the strengths even of these scaffolds should be sufficient to ensure successful fixation. Whilst structures such as this are unlikely to be significantly load bearing they may well provide a suitable template for aligned soft tissue regeneration and repair. Further biological characterisation, biomechanical testing and in vivo studies would clearly be required in order to substantiate this.

Conclusions

Composite collagen based structures have been produced with axially aligned collagen fibres and a pore structure generally recognised as favourable for cellular migration, proliferation and attachment. Significant porosity existed within fibres that were used in composite formation without prior cross-linking and this resulted in a reduction in the tensile properties compared with fibres that were cross-linked prior to use. Channels were also observed around the fibres that were not previously cross-linked. Whilst the higher mechanical strengths observed with previously cross-linked fibre bundles are clearly desirable, pore channels may enhance cell migration and aligned tissue regeneration.

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Figure Captions

Figure 1: The fibre extrusion process: (a) schematic of the experimental set-up, (b) fibre bundles wound onto spools, (c) fibre bundle after drying and removal from the spool.

Figure 2: Photographs of upper and lower surfaces of freeze-dried composite samples with: (a), (b) fibre bundles used with previous cross-linking and (c), (d) without previous cross-linking.

Figure 3: SEM images of composite material; (a) – (c) show material produced where cross-linking of the fibres was carried out prior to casting of the matrix, (d) - (f) where cross-liking of the fibres was not carried out.

Figure 4 (a), (b), (c) – optical micrographs of non-crosslinked fibre sections dry, after 10 minutes soaking and after 1 hour soaking respectively; (d), (e), (f) – optical micrographs of crosslinked fibre sections: dry, after 10 minutes soaking and after 1 hour soaking respectively; (g) variation in % swelling (as defined by change in diameter for fibres with and without crosslinking).

Figure 5: Tensile stress as a function of strain for: (a) composite material with cross-linked fibre bundle, (b) composite sample where cross-linking of the fibre bundle was not carried out prior to matrix addition. Different colours correspond to repeats

Figure 6: Photographs of composite samples during tensile testing: (a) and (b) show failure of composites containing fibre bundles with previous cross-linking and (c) with fibre bundle without previous cross-linking.

Figure 7: Compressive stress as a function of strain for: (a) composite material with cross-linked fibre bundle, (b) composite sample where cross-linking of the fibre bundle was not carried out prior to matrix addition. Different colours correspond to repeats.

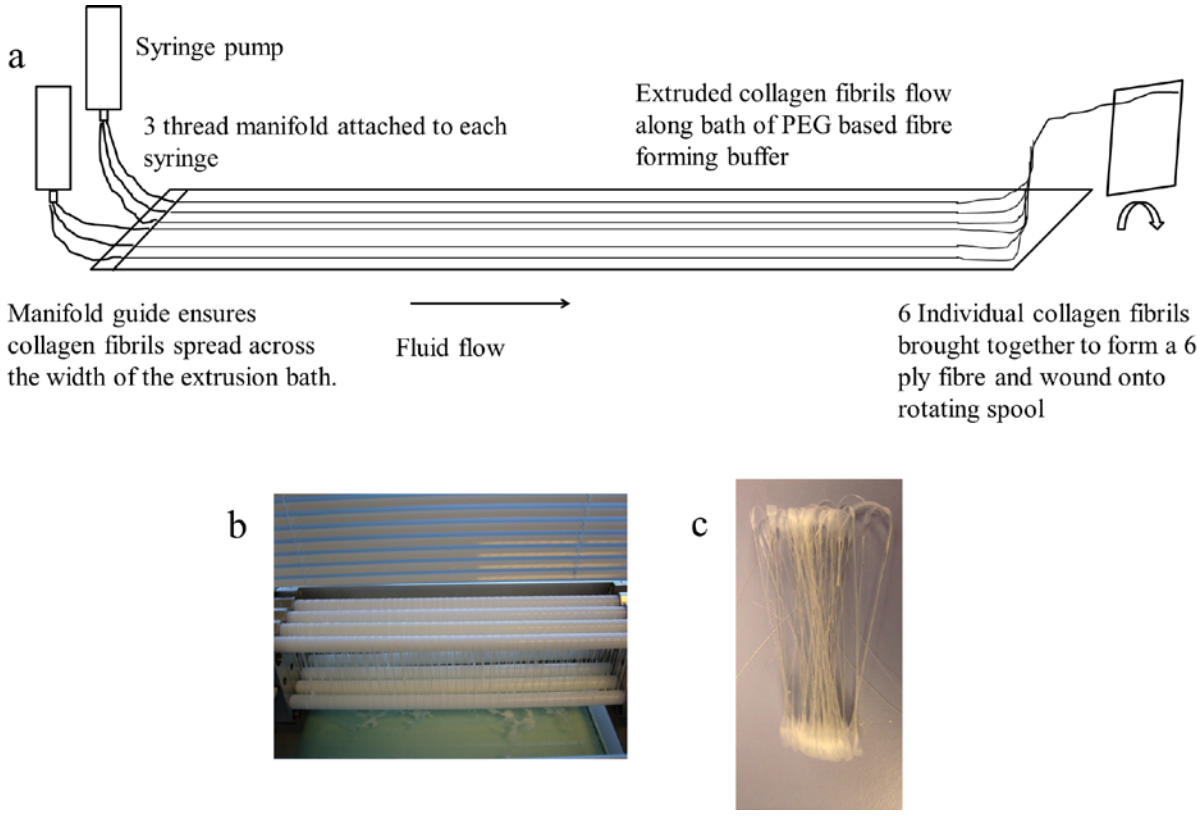
a
Syringe pump
3 thread manifold attached to each syringe
Extruded collagen fibrils flow along bath of PEG based fibre forming buffer
Manifold guide ensures collagen fibrils spread across the width of the extrusion bath.
Fluid flow
6 Individual collagen fibrils brought together to form a 6 ply fibre and wound onto rotating spool
b
c

Figure 1

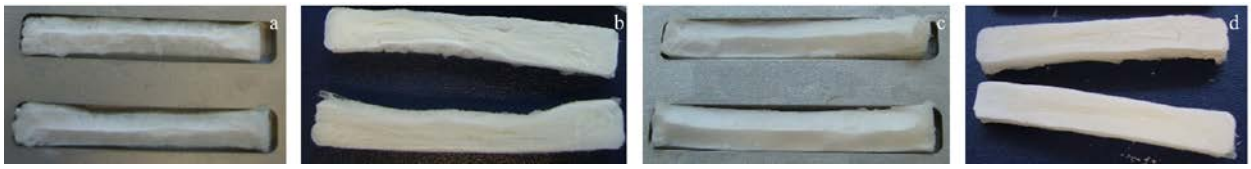

Figure 2

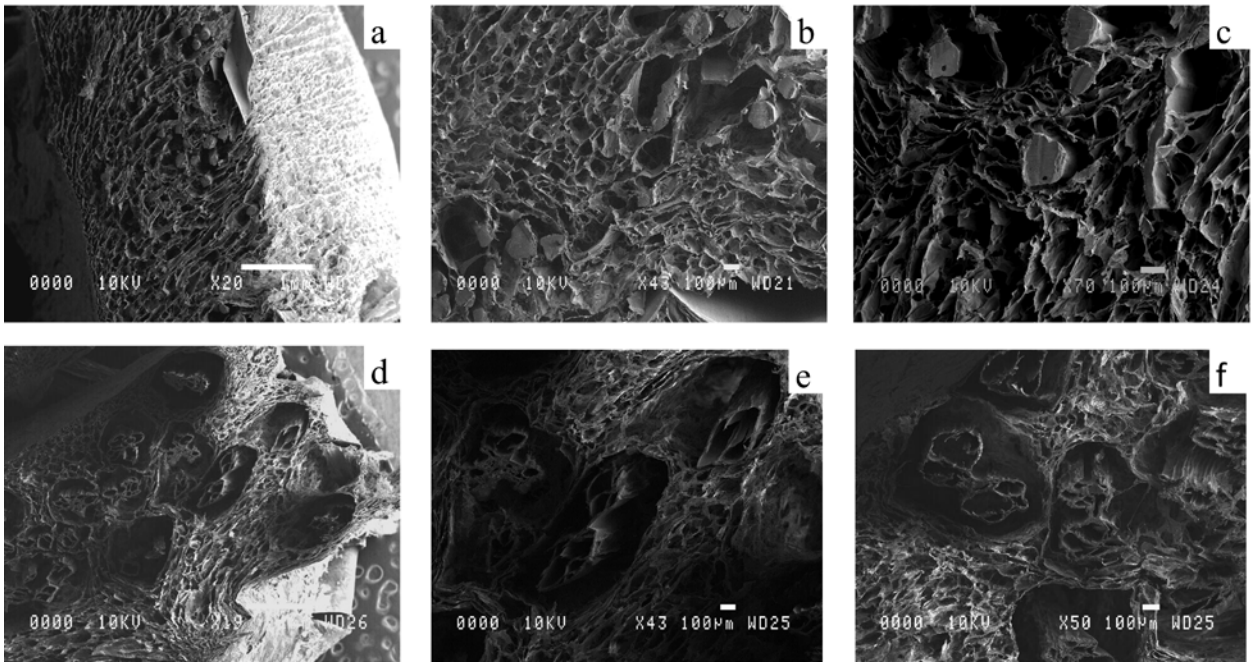
a
0000 10KV X20
b
0000 10KV X43 100μm WD21
c
0000 10KV X70 100μm WD24
d
0000 10KV X19 WD26
e
0000 10KV X43 100μm WD25
f
0000 10KV X50 100μm WD25

Figure 3

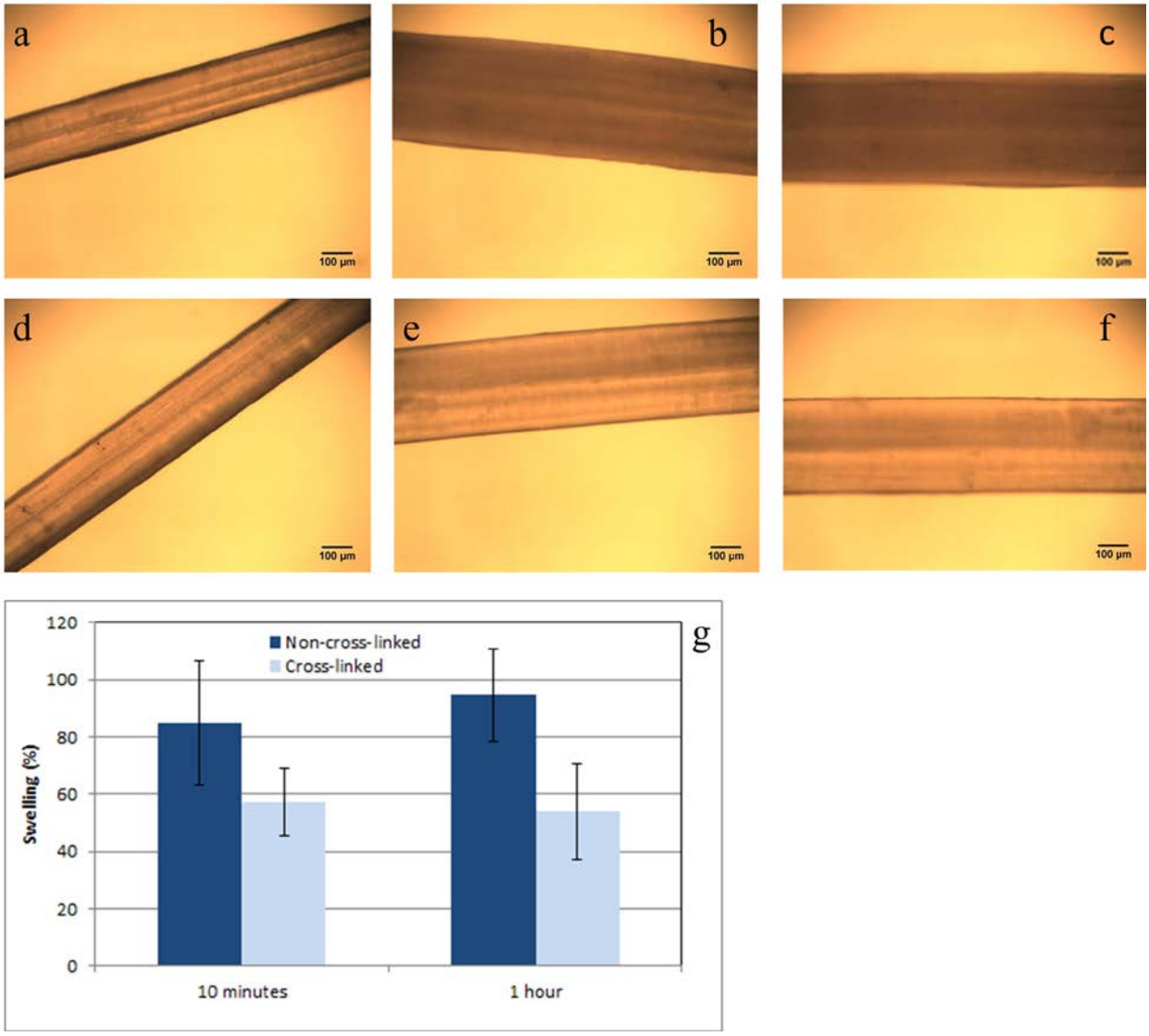
a
b
c
d
e
f
100 µm
g
Non-cross-linked
Cross-linked
Swelling (%)
120
100
80
60
40
20
0
10 minutes
1 hour

Figure 4

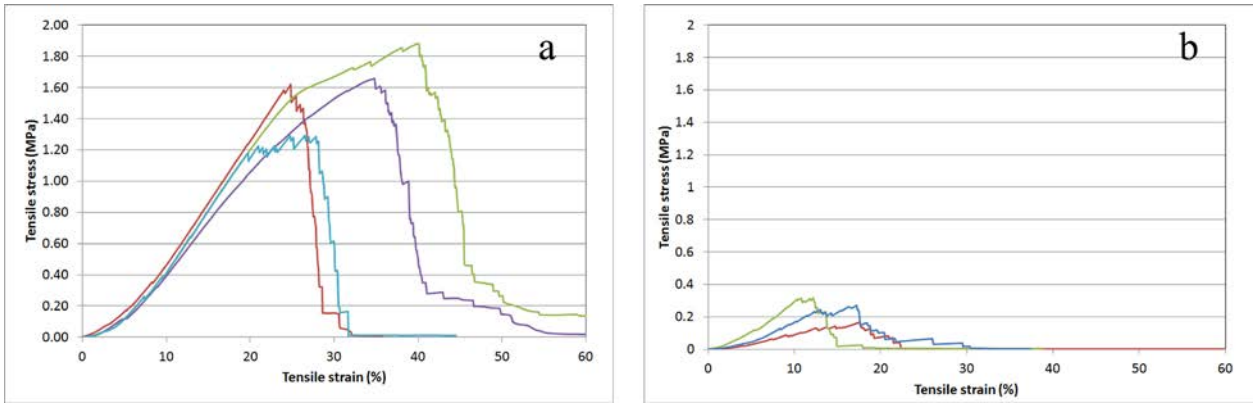
a
Tensile stress (MPa)
Tensile strain (%)
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0
10
20
30
40
50
60
b
Tensile stress (MPa)
Tensile strain (%)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60

Figure 5

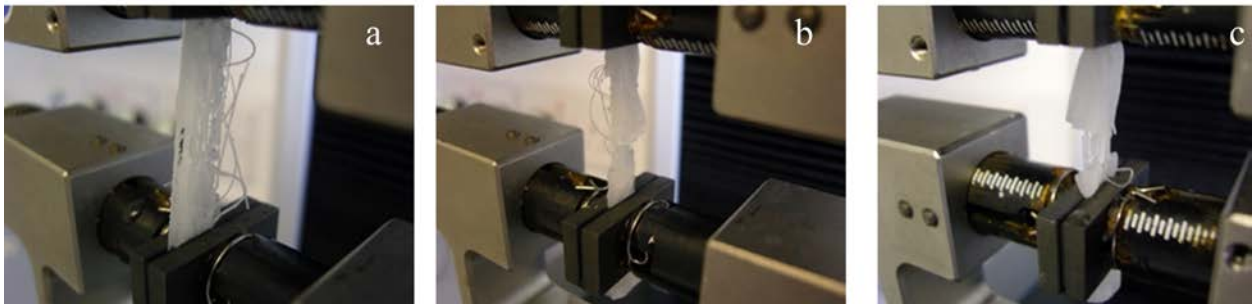

Figure 6

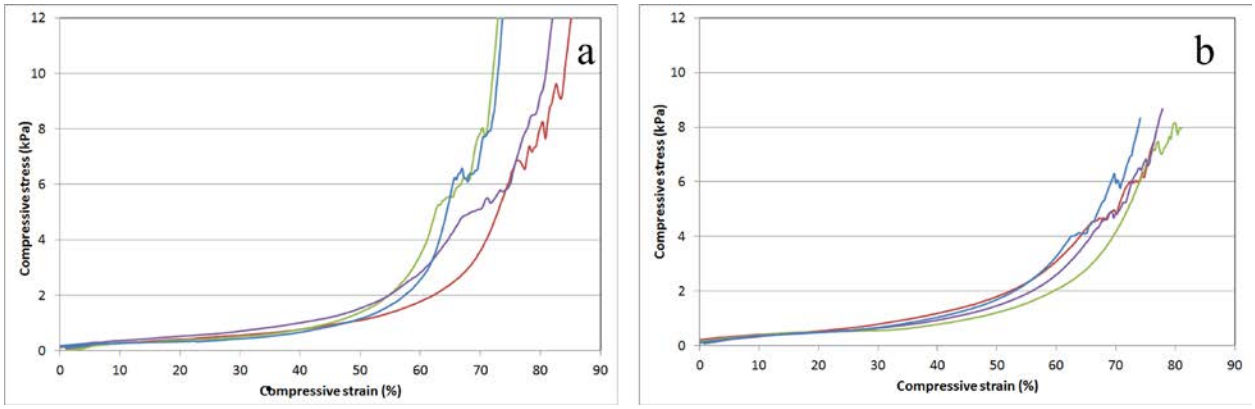
a
b
Compressive stress (kPa)
Compressive strain (%)
0
2
4
6
8
10
12
0
10
20
30
40
50
60
70
80
90

Figure 7

Sample	Load to failure (N)	UTS (MPa)	Strain to failure (%)	3-5% strain modulus (MPa)
With cross-linking	61.94 (15.54)	1.55 (0.30)	30.17 (7.17)	3.89 (0.35)
Without cross-linking	11.75 (2.62)	0.24 (0.08)	15.40 (3.22)	1.59 (1.03)
Matrix only	0.64 (0.25)	0.015 (0.0061)	11.65 (3.48)	0.11 (0.051)

Table 1: Tensile properties of composite samples using fibre bundles with and without prior cross-linking. Mean values are given with the associated standard deviations in brackets.

Material	**Low strain modulus (kPa)**	**High strain modulus (kPa)**
Cross-linked	1.80 (0.63)	17.29 (8.02)
Non-cross-linked	2.04 (0.31)	14.78 (3.98)
Matrix only	3.48 (0.93)	9.38 (7.26)

Table 2: mean compressive moduli for composites containing fibre bundles with and without prior cross-linking as well as matrix only for comparison, standard deviations are shown in parenthesis. Low strain modulus was determined at 10-15% strain and high strain modulus at 55-60% strain.