The use of novel electrospun scaffolds in musculoskeletal tissue engineering: a focus on the rotator cuff

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Introduction

The ageing worldwide population has put degenerative pathologies under the spotlight for scientists and clinicians. This is particularly true of musculoskeletal disease, where rising treatment expenses, ADL disruption and lost workdays (8.8 million workdays are lost annually in the UK) are a significant and growing burden to global economies.

Within musculoskeletal disease, ligament and tendon pathology remain incompletely understood and inadequately treated. Rotator Cuff (RC) tears affect 15% of 60 years olds, increasing to 30% of 80 years olds⁶¹. Even after optimal non-operative management, many patients progress to surgery. In 2015, there were 400,000 such surgical repairs in the USA and 10,000 in the UK, representing a 700% increase over the preceeding decade. However, recent data from the UKUFF trial indicates that surgical procedures fail in 40% of patients within 12 months regardless of technique (unpublished data), with other studies reporting failure rates as high as 94%¹⁶.

Current repairs involve suturing damaged tendon back to bone and often attempting to augment the repair with an artificial or biological scaffold. These scaffolds are designed to provide mechanical support but do not actively help regenerate the underlying tendon. Conversely they may produce inflammation, foreign body reactions and fistula formation, ultimately increasing failure rates. Scaffolds are predominantly decellularised matrix from animal xenografts and human cadaveric allografts; but synthetic polymer products are also used. Commercially available patches are relatively homogenous in structure, shape and surface morphology, not reflecting the complex transition zone from tendon to bone tissue, or the nanoscale topographic features of tendon known to influence resident tenocyte behaviour.

The growing market pressure and current limitations in RC repair strategies have spurred interest in a Tissue Engineering/Regenerative Medicine solution to assist in soft tissue repair.

‘Tissue Engineering/Regenerative Medicine’ (TERM)

TERM strategies consist of one or more components of the tissue engineering triad (Figure 1):

1. ***Bioactive molecules and/or growth factors.***

The importance of these molecules stems from the greater understanding of their role in tendon development and potential role in influencing differentiation of pluripotent cells

towards a tenocytic lineage. One such factor, Fibroblast Growth Factor (FGF) induces Tendon Progenitor Cells (TNPs) within the embryo through the action of Scleraxis (Scx)⁵², a transcription factor gene specific to differentiation towards TNPs^{47,64}. FGF has been shown to mediate repair in rotator cuff tears¹¹ by stimulating cell proliferation and migration in addition to fibroblastic collagen production, and angiogenesis at injury sites^{11,58,62}. Moreover, *in vitro* studies have also shown potential for FGF and Growth Differentiation Factor (GDF) factors in increasing the speed and strength of tendon healing^{12,25,60}. These factors may have a supplementary role in tendon repair.

Transforming Growth Factor β (TGF β), is another molecule which has been shown to be integral in expression of Scx and subsequent genesis of tendons in the embryo. Pryce *et al.* found that Tgfb2(-/-) and Tgfb3(-/-) double mutant murine embryos did not develop tendons or ligaments in the limbs, trunk, tail and head⁴⁷. Further evidence within canine models has shown that combinations of Insulin-like Growth Factor (IGF-1) and TGF β 1⁵¹ and that TGF β 3²⁷ within a 3D structure, can aid in differentiation of MSCs towards a tenogenic lineage.

2. *Cells*

Cell populations utilised for TERM can be categorised into stem cell (predominantly MSCs and increasingly iPSCs) and differentiated cell populations.

MSCs are stem cells capable of differentiating into connective tissue phenotypes such as bone, cartilage, tendon, muscle and fat. They are found in bone marrow or adipose tissue and offer a plentiful source for tissue engineering. There is a paucity of evidence that MSCs alone can regenerate tissues and contrarily, work by Gulotta *et al.* in murine models has shown that adding unaltered MSCs to healing RC entheses has no effect in collagen fibre organisation or the mechanical properties of repaired tendon¹⁷. However, work from the same group has shown that by manipulating MSCs with viral vectors for certain genes such as MT1-MMP¹⁸ (upregulated during embryogenesis at the tendon bone enthesis) and Scx¹⁹, RC repairs have higher load and stress to failure values in addition to higher stiffness with increased fibrocartilage volume on histology. In the context of scaffolds, Yokoya *et al.* have shown that MSCs in the presence of a PGA

sheet, can improve the strength of repair compared to a scaffold or traditional repair alone⁷².

No up-to-date review would be complete without acknowledging the recent paradigm shift reframing MSCs as more than sources of terminally differentiated cells, but rather trophic and/or immunomodulatory cells, regenerating host cells⁵⁵. Work in this field, in the musculoskeletal context has yet to be explored. Moreover, tendon progenitor cells have recently been isolated with characteristics of “clonogenicity, multipotency and self-renewal”⁷, although research is still in its infancy, these cells have the potential to act as a source of tenocytes.

Native cells have been investigated as tissue engineering treatments (autologous tenocyte implantation) and randomised controlled trials are currently underway to determine efficacy²². Case reports have shown positive results⁶⁶. Barriers to such therapies include the limited availability of donor sites, donor site morbidity, reduced replication rates and functionality of aged cells and potentially lengthy *in vitro* culture expansion times with associated phenotypic drift⁷⁰.

3. *Scaffolds*

Physical frameworks, either biologic and synthetic, are important in providing structural integrity and mechanical properties to tissues immediately after repair, ideally matching those of the tissue they are implanted in. Scaffolds can also contribute an intricate 3D environment for cells at a nano scale. This is important for tendon regeneration as mature tendon has a highly organised hierarchical structure down to the collagen fibrils (100-200nm). Scaffolds can provide nano scale biophysical cues to promote cell differentiation and matrix production²⁰. Furthermore, scaffold matrix chemistry influences cell adhesion to the matrix⁴⁹ and migration into it⁵³.

Electrospinning

Electrospinning is an attractive technique for tendon TERM biomaterial production as it allows fabrication of fibres similar in size and alignment to those occurring in natural extracellular

matrix^{46,57}. Electrospun scaffolds can also be easily modified to manipulate the scaffold mechanical, biological and physical properties, potentially explaining the significant increase in electrospinning in MSK research over the past decade (Figure 2)

Basic electrospinning (figure 3A) involves injecting a solution into an electric field. The electric field stretches the solution towards a collector. When the electrostatic force overcomes the surface tension of the solution, the solution erupts towards the collector (Figure 3B). Whilst travelling to the collector, the volatile solvent evaporates leaving solid fibres distributed on the collector.

Electrospinning advancements

Pre-fabrication

Solvents and salts can be added to change the viscosity and dielectric constant of the solution and consequently the behaviour during electrospinning^{24,59,65}. Molecules can also be combined through direct blending so they are incorporated into the fibres during electrospinning. Drugs to influence cell response^{68,75}, nanomaterials to alter mechanical properties⁵⁴ (He 2014, Kolluru 2014) as well as DNA plasmids and siRNAs to manipulate gene expression have been successfully combined^{9,37}.

During fabrication

During the electrospinning process, modifications of electrospinning variables can alter the size, alignment and density of the fibres. It is also possible to spin from multiple spinnerets to combine fibres of different materials or sizes. Additionally, cells have been included in the electrospinning process, transforming a scaffold from an inductive or conductive scaffold to an independently autogenic tissue⁶³.

Post-fabrication

After electrospinning, fibres can be cross-linked⁵ and annealed³³ to alter mechanical properties or undergo other surface treatments such as salt leaching⁴¹ to further modify the surface morphology and biological activity of the materials.

Modified Electrospinning Techniques

A number of advanced electrospinning techniques have been developed to further enhance electrospinning's capabilities. Melt Electrospinning uses molten polymers to allow electrospinning of semi-crystalline polymers not suitable for traditional solvent based spinning. Emulsion electrospinning allows inclusion of proteins and hydrophilic molecules not amenable to direct blending in organic solvents. Low Temperature Electrospinning results in ice crystals forming between fibres which on melting leaves an increased distance between fibres. Co-axial spinning feeds two solutions into the electric field through a modified spinneret to allow generation of fibres with different surface and core materials.

Materials and Methods

Search strategy and criteria (Figure 5)

A study protocol was developed and a literature search of the MEDLINE/PubMed database was conducted between December 2015 and January 2016 using the search string ‘electrospinning AND tendon’.

Exclusion criteria applied to studies were: publication date greater than five years, non-English language publications, review articles and non-musculoskeletal cell-type studies. Studies satisfying inclusion and exclusion criteria were independently reviewed by two of the authors (NSN and ETS).

Outcomes of search

Of 38 initial studies, eighteen were included in the systematic review (Table 1). All studies were basic science in nature, with seven (38.9%) reporting animal *in vivo* work and none with human clinical data.

Varying outcome measures were reported and common themes identified. These included (1) using electrospinning technology for drug delivery, (2) use of novel and biological materials in manufacture of scaffold, (3) increased mechanical strengths of materials, and (4) techniques to improve the nanotopographical properties of scaffolds.

Results

Drug Delivery

The incorporation of drugs into scaffolds and the drug release profiles were the predominant outcome measure of several studies^{26,34,36,76}. Techniques employed to incorporate the molecules into electrospun scaffolds were varied and included dissolving drugs directly into the electrospinning solution (bFGF⁷⁴, naproxen³⁶ and hyaluronan) or producing an emulsion with hydrosols (mitomycin C [MMC]). One study made use of bFGF-loaded nanoparticles for drug release³⁴.

Lui *et al.* used PLLA and PCL dissolved in HFIP to produce different scaffolds with a constant proportion of naproxen. They found that increasing the proportion of PLLA:PCL in the electrospun substrate was negatively correlated to percentage release of naproxen; moreover, by using water as a co-solvent to HFIP, sustained drug release up to three weeks was achieved. Seeded murine cells lines indicated that naproxen had no effect on cell viability or morphology.

Zhao *et al.* incorporated bFGF incorporated into scaffolds, noting a burst release of 15.4% over the first 48 hours with subsequent release for a further 22 days. They also demonstrated human dermal fibroblasts seeded onto such scaffolds proliferated faster and maintained phenotype more than cells seeded on non-bioactive control scaffolds.

Further work by Liu *et al.* compared bFGF incorporated in dextran glassy nanoparticles to bFGF included directly in the electrospinning process. They found that nanoparticles facilitated a steady release pattern with no burst release, of greater duration (30 vs 20 days) and with a higher cumulative release of drug (6400pg vs 2900pg) than non-nanoparticle controls. Both studies using bFGF demonstrated decreased adhesion formation and improved collagen organisation and mechanical properties in murine models.

In scaffolds with MMC included as hydrosols, a burst with 26-31% of the drug was seen in the first 48 hours with a subsequent tailoring off after the next six days. MMC concentration was indirectly related to cell viability, proliferation and a tenocytic phenotype. In rat Achilles and rabbit FDP models, MMC hydrosol scaffolds reduced inflammation and peritendinous adhesions with the improved fibre orientation⁷⁵.

Liu *et al.* incorporated hyaluronic acid (HA) into PCL scaffolds. Gradual release patterns were observed after initial bursts, especially at 4 and 8% HA. By increasing the proportion of HA,

the tensile strength of the scaffolds fell (from 2.13 to 1.55MPa as HA percentage increased from 0 to 12%). Conversely the tensile moduli increased as the HA percentage increased. In vitro models demonstrated that pluripotent murine stem cells attached to PCL/HA scaffolds and proliferated more as HA percentage composition increased. A galline tendon-healing model demonstrated that at 12%HA/PCL, scaffolds reduced the severity of peritendinous adhesions and increased tendon healing scores compared to PCL only scaffolds³⁵.

Clinical applications

Novel methods to aid in vivo studies have been proposed. Martin *et al.* described a method by which scaffolds could be studied post-implantation through radiographic imaging; they introduced zirconium(IV) oxide nanoparticles molecules into electrospun solution, with good biocompatibility found in vivo murine intervertebral disc and ex vivo bovine meniscal tear models³⁹. Specifically, they found good overlying fibrous but not calcific deposition. However, in vitro studies found that metabolic activity of bovine MSCs seeded on the scaffolds was lower than that of TCP. Importantly, microCT was used to visualise the implants, which has directly transferable benefits for patients, where healing can be monitored, migration and impingement can be detected and scaffolds can be localised should any revision be required.

Biodegradable materials

Cellulose, gelatin, hyaluronan, chitosan, silk and digested animal tendon tissue used as components in the production of scaffolds over the review period. Zhang *et al.* found that a combination of chitosan and gelatin produced scaffolds via a Stable Jet Electrospinning (SJES) technique, were of a more linear morphology and, increased tensile and greater elasticity than controls produced by conventional electrospinning techniques⁷³. The SJES scaffolds had a tenogenic influence on human iPSCs with in vitro models demonstrating a fibroblastic phenotype, tenogenic gene expression, and non-osteogenic phenotype with no variation in cell proliferation rates compared to controls. An in vivo murine Achilles tendon model, with human iPSCs seeded into the tendon, showed SJES scaffolds increased the spindle morphology of cells, number of collagen fibres and collagen fibre size (27.8% larger than controls). Similar to *in vitro* work, IHC showed increased tenogenic and decreased osteogenic gene expression. Furthermore, mechanical properties (increased stiffness, failure force, stress at failure and Young's modulus) were improved in the SJES group.

Chainani *et al.* used mechanical and chemical techniques to digest porcine tendon pulverised tendon tissue into a powder and coated this onto a PCL electrospun scaffold¹⁰. Increased yield strength was found after 28 days of culture with human ASCs, in addition to indications of cells

division, matrix deposition (increased collagen 1), and tenogenic gene expression, more so than controls without tendon coating.

Silk fibroin was integrated into scaffolds in two different manners. Yang *et al.* made use of dissolving degummed silk into chemical solvent and blending this to form an electrospinning substrate⁶⁹; whereas Naghashzargar *et al.* electrospun conventional materials (e.g. PCL) onto silk fibroin yarns⁴⁰. Silk substrate scaffolds and silk fibroin electrospinning techniques, which were seeded with murine BMMSCs and murine fibroblasts respectively, showed no change in cytocompatibility and improved mechanical properties.

Mechanical Properties

The common themes to augment electrospun scaffold mechanical properties were the formation of nanoyarn scaffolds and the addition of nanoparticles.

Yang *et al.* tested three electrospun silk and PCL scaffolds; (1) aligned nanofibrous scaffolds, (2) random nanofibrous scaffolds and (3) Nanoyarn Re-Inforced Scaffolds (NRS)⁶⁹. The Young's modulus, tensile strength and elongation at break were evaluated both parallel and perpendicular to the direction of fibre alignment and at 90 degrees for the randomly orientated scaffold. For the aligned scaffold this equated to Young's moduli values of 433.56MPa (parallel) and 29.72MPa (perpendicular) and for the NRS 288.95MPa and 32.51MPa. No difference was noted for the randomly aligned scaffold. The NRS and aligned scaffolds also had significantly greater tensile strengths in the parallel compared to perpendicular direction. Conversely the elongation at break values were significantly less in the parallel than perpendicular directions, 58.78% (parallel) and 212% (perpendicular) for the aligned scaffold and 40.82% and 162.76% for the NRS.

Xu *et al.* performed similar assessments of their nanoyarn scaffold compared to aligned and random scaffolds⁶⁷. They did not specify if this was done parallel or perpendicular to fibre orientation. They do however note the nanoyarn scaffold had a significantly lower tensile strength and elastic modulus but higher elongation at break value.

He *et al.* used CNCs at 20% w/v to increase the tensile strength of the CNC-incorporated electrospun scaffolds from 16.6MPa to 33.6MPa and the elastic modulus from 0.69GPa to 1.87GPa²¹. Cell testing showed no toxic effects from the use of CNCs and cell attachment and spreading patterns consistent with healthy primary human dental follicle cells

Sheikh *et al.* added MWCNTS at 0.06, 0.33 and 0.66% w/v to increase the tensile stress of scaffolds from 11.4MPa without MWCNTS to 39.6MPa, 51.23MPa and 32.99MPa respectively⁵⁴. Of note, at 0.66%, the MWCNTs were improperly distributed preventing uniform stress distribution and likely resulting in the observed inferior tensile strength. In vitro testing found no negative effects of MWCNT addition on cell behaviour or attachment.

Surface architecture

A number of studies examined altered surface topography as a means to enhance tendon repair. A common strategy was to increase porosity and thus the ability of cells to infiltrate throughout the scaffold, as well as facilitate nutrient and waste exchange.

Yang *et al.* increased porosity through the formation of nanoyarns. The NRS had yarns with diameters of 30.56µm, comprised of nanofibres with a mean diameter of 868.94nm. Their NRS had a significantly greater pore size (551.41µm) compared to their aligned (16.7µm) and random nanofibrous scaffold (72.8µm) controls⁶⁹. The porosity of the NRS was higher at 79.2% compared to 75.1% and 73.6% for the random and aligned scaffolds. This increased porosity allowed improved murine MSCs cell infiltration in in vitro testing. MSCs reached 150µm into the scaffold by day 7, 300µm by day 14 and 500µm by day 28 compared to 40µm at day 28 on randomly aligned scaffolds and no cell infiltration on aligned scaffolds after 28 days.

Xu *et al.* manufactured nanoyarn scaffolds with a mean pore size of 28.5µm compared to aligned (4.43µm) and randomly aligned (4.31µm) scaffolds⁶⁷. The porosities were reported as 85.9% for the nanoyarn scaffold compared to 73.9% and 74.1% for the random and aligned scaffolds respectively. Primary rabbit tenocytes seeded on to the nanoyarn scaffold were able to better infiltrate the scaffold over 14 days of culture compared to cells seeded onto aligned and random nanofibre controls. This finding was supported by significantly higher cell counts on their nanoyarn scaffold at 7 and 14 days culture compared to controls. rtPCR showed primary tenocytes seeded on the nanoyarn scaffold expressed significantly higher levels of collagen 1, collagen 3, Decorin, Tenascin C and Biglycan mRNA compared to cells seeded on random and aligned nanofibrous scaffolds.

Beason *et al.* fabricated PCL scaffolds of increasing porosity using a sacrificial fibre technique. This involved co-spinning a PCL-PEO scaffold⁶. When submerged in ethanol, the soluble PEO dissolves leaving solely PCL fibre scaffolds. The vacant space left by PEO fibres increases the porosity compared to the controls spun using PCL alone (Baker *et al.* 2008⁴). The high and low porosity scaffolds were then tested in a murine rotator cuff model. At 4 and 8 weeks in vivo,

cell infiltration and colonization were lower in the sacrificial high porosity PEO/PCL scaffold compared to the less porous PCL scaffold.

On the nanoscale, fibre alignment can be manipulated to alter tenocyte behaviour. Orr *et al.* tested multi-layered aligned nanofibre scaffolds against multi-layered random nanofibre scaffolds⁴⁴. The mean fibre diameter of aligned and non-aligned scaffolds was not significantly different at 1.76µm and 1.57µm respectively. The fiber alignment index of aligned scaffolds was 17 times greater than that for non-aligned scaffolds. Both scaffolds supported hASC growth with cells invading through the full thickness of the scaffolds. However collagen deposition was more abundant in aligned scaffolds when assessed by Picrosirius red staining. Furthermore COL3A1 and TNMD mRNA expression was higher on aligned scaffolds. After 28 days of culture, aligned scaffolds demonstrated greater increases in Young's modulus and yield stress than those observed in non-aligned scaffolds.

Samavedi *et al.* fabricated scaffolds of altering alignment to engineer a scaffold more suitable for replicating the complex transition between bone and tendon for use in patella tendon reconstruction⁵⁰. Using three different spinnerets and shields, a three-zoned scaffold was fabricated to increase biomimicry of the native bone-tendon-bone junction for patella tendon repair. A 3 part scaffold was fabricated with random PLGA fibres (mimicking bone) transitioning to aligned PCL fibres (mimicking tendon), with the PCL fibres again transitioning back to the random PLGA fibres. The PLGA sections had a tensile modulus of 24-28MPa, three times that of the PCL sections, better replicating the nanoenvironment of bone. Culture of MSCs on the scaffold showed aligned cells with high aspect ratios on the aligned PCL component (similar to tenocytes) and polygonal randomly orientated cells on the unaligned PLGA section (similar to osteoblasts).

Discussion

This review of literature highlights the current directions under investigation and consideration for tendon TERM research regeneration and can aid researchers.

Drug delivery

As drugs are developed for more specific molecular targets, there is an increased need to prevent adverse systemic side effects, whilst lengthening the half-life and effect in the targetted pathological tissue. Nanotechnology⁴⁵, liposome³ and polymeric micelle²⁹ technologies have all been considered for drug delivery, though none of these mechanisms would have the initial proximity and potential longevity an electrospun scaffold implanted within target tissue could.

We found five original research papers looking into delivery of naproxen, celecoxib, Mitomycin C (MMC) and growth factors such as bFGF^{26,36,67,74,76}. These molecules were investigated secondary to previously published work suggesting potential efficacy in tendinopathy. Although primarily used as an anti-tumour drug, Mitomycin C (MMC) has been shown to inhibit adhesion formation through upregulation of the apoptotic gene Bax within fibroblasts³⁰ in animal tendon repair models. Toxicity and severity of side effects have previously limited the use of the drug within a scaffold, as release profiles were unsafe. Similarly, naproxen (an NSAID) has been shown to prevent adhesion formation and reduce inflammation, leading to it being a commonly prescribed therapy for tendinopathy. Furthermore, celecoxib, a selective COX-2 inhibitor acts to inhibit fibroblast proliferation and collagen expression by inhibiting ERK1/2 and SMAD2/3 phosphorylation of the cells. Finally, having its efficacy through angiogenesis and propagation of an anabolic phenotype, bFGF is a growth factor that has previously in vitro and in vivo been shown to improve tendon repair.

In addition to anti-adhesion and anti-inflammatory molecules, future targets include vascular proliferation after Bjur *et al.* discovered an association between increased neovascularisation of tendon and pain in tendinopathy^{1,8}. Neural pathways in painful tendinopathy were associated with vascular structures within Achilles tendon biopsy samples. This prompted pilot use of polidocanol (a sclerosing agent) injection in patients with painful tendinopathy. The initial study found an 80% patient satisfaction

rate and significantly reduced Visual Analogue Score (VAS) for pain (from 74 to 8), and a marked reduction in vascularisation in the Achilles tendons of this group⁴²; these findings have been substantiated in a double-blinded RCT of twenty patients², though it should be noted that the benefits were not conferred in a separate RCT for patellar tendinopathy²³. Polidocanol has been shown to be compatible with incorporation into an electrospun scaffold, though cytocompatibility and mechanical properties have yet to be determined⁴⁸.

Biodegradable materials

Use of biodegradable materials in electrospinning has increased recently for a number of practical benefits. Although their beneficial properties vary according to source, biodegradable materials can be biocompatible, non-immunogenic/inflammatory (bioinert) and have low toxicity profiles. Some, such as polymers of hyaluronan, have additional bioactive properties such as a conducive role in ECM remodelling; angiogenesis, and influencing wound healing, cell proliferation and motility. When incorporated into, or being the chief constituent of electrospun scaffolds, the biological merits of biodegradable materials are synergised to a larger scale and can be arranged in a manner to influence cell morphology in addition to biological cues.

Mechanical properties

While electrospun materials may induce favourable cell and tissue behaviour to augment tendon healing, their baseline mechanical properties are often inadequate for tendon repair. As the mechanical environment is crucial for successful tendon repair, techniques to enhance the mechanical properties have advanced over the last decade to harness the favourable biological influences of electrospun fibres into a robust scaffold suitable for surgical implantation. These include altering the polymer material used for electrospinning, combining different fibre types, adding molecules, multi-layered scaffolds, multi-component scaffolds and post fabrication treatments such as annealing and cross-linking – strategies which have seen varying degrees of success.

One prominent strategy over the past 5 years was the use of nanoyarn based scaffolds. The aggregation of fibres into yarns significantly increased the mechanical properties

of nanoyarn scaffolds above comparable nanofibre scaffolds. Both Yang and Xu *et al.* claim to achieve a mechanical strength sufficient for tendon repair through this technique. Another theme to increase mechanical properties was the addition of nanoparticles to the electrospun material, with He *et al.* using cellulose nanocrystals (CNCs) in a cellulose electrospun scaffold and Sheikh *et al.* adding multi-walled carbon nanotubes. These papers suggest that significant augmentation of mechanical properties through nanoparticle addition is achievable without cytotoxicity or compromising cell attachment and behaviour. However, neither of these studies formally assessed gene expression or quantified protein expression in scaffolds with the added nanomaterials. A negative influence on tenocyte behaviour and gene expression could result in inferior clinical outcomes even with adequate cell attachment and proliferation.

Surface architecture

Originally ECM was thought to provide physical and spatial support for the embedded cell population, with cell behaviour driven largely through molecular clues from nearby or distant cells and cell-cell interaction. However, with the ability to fabricate scaffolds and materials with deliberate variances at the nanoscale, the importance and influence of cell-matrix interactions is becoming more apparent. This interaction is bidirectional, with cells initiating ECM changes and ECM changes altering cell behaviour. This dynamic interaction, termed Biophysical Epigenetic Regulation (BER), is involved from stem cell differentiation ¹⁴ through to carcinogenesis ³⁸. BER occurs in biological and synthetic nano-topographies ^{13,56}. Significantly for RCT repair, cell-scaffold interactions have enhanced tenocyte repair mechanisms, matrix deposition and cell phenotype ^{28,77,15,32,31,43,71}. Harnessing these cues into a tendon repair scaffold could significantly improve rotator cuff repair outcomes.

We have identified a number of studies examining altered surface topography to enhance tendon repair. One strategy was to increase porosity and thus the ability of cells to infiltrate throughout the scaffold, as well as assist in nutrient and waste exchange. Increasing porosity was achieved in a variety of methods including nanoyarn formation (Yang *et al.*, Xu *et al.*), multilayered scaffolds (Orr *et al.*) and

sacrificial co-spun scaffolds where one fibre population was removed by submersion in ethanol prior to experimentation (Beason *et al.*).

In addition to increasing cell invasion and proliferation, Xu and Yang *et al.* showed a support of tenocyte phenotype in nanoyarn scaffolds in vitro. Faster cell infiltration, higher cell counts and increased tendon matrix ECM gene expression are suggestive of a scaffold which would facilitate tendon repair.

Beason *et al.*'s work, however, shows extrapolation from in vitro to in vivo results is complicated. The reduced infiltration observed in vivo on the lower porosity scaffold was the opposite of previous in vitro and subcutaneous in vivo models suggesting the advantage of increased porosity may be negated through poor handling or poor implantation practices or when placed under pressure and strain in vivo in real world situations. Engineering porosity into a scaffold which can handle the challenges of implantation and in vivo function requires further work.

Manipulating the nano-architecture had considerable influence as shown by Orr *et al.* and Samavedi *et al.* Increasing fibre alignment promoted collagen deposition, mechanical strength and ECM gene mRNA expression in hASCs. Differing fibre alignment and mechanical properties in PCL and PLGA areas within a single scaffold were able to promote MSC shape changes consistent with differentiation down tendon and bone lineages. Unfortunately no gene expression or further cell characterization data were published to support these achievements. However the cell shape changes suggestive of differentiation down two cell lineages in morphologically distinct scaffold areas are a testament to the increasing complexity and promise of electrospun biomaterial design.

Limitations

Many studies have used biodegradable materials for obvious reasons. However the in vitro and in vivo models have not assessed the effects of degradation. Degradation will impact on the mechanical properties weakening the scaffold significantly. Exploration of the timeframes over which this occurs is crucial so it can be matched to the rate of tendon tissue deposition and maturation to ensure the mechanical

strength of the repair is always great enough to prevent breakdown.

In scientific research, extrapolation of *in vitro* to *in vivo* results is difficult. The lack of representative *in vitro* tissue testing and *in vivo* models in the studies reviewed further complicates this. Specifically *in vitro* human tissue/cell cytocompatibility testing was conducted in less than 15% of the fifteen papers. Furthermore *in vivo* testing was limited to murine and galline models, known to not reflect the larger forces acting in human tissues.

These difficulties were highlighted by Beason *et al.*'. *In vitro* data suggested the more porous scaffolds would allow better cell infiltration, ECM deposition and mechanical properties when implanted in a rotator cuff tear model, however the opposite was observed. Consequently many of these scaffolds, whilst showing significant promise *in vitro*, will need significantly more research and experimentation with human tissue, both *in vitro* and *in vivo*, before a new generation of electrospun biomaterials can be incorporated into clinical practice.

With all drug delivery system experiments, cytocompatibility was another metric of success for these drugs, although limited human cell-line testing was performed, and influence of fibroblast phenotype and viability were contradictorily used as a positive and negative marker of success^{26,36,67,74,76}.

Conclusion

The optimal bioengineered scaffold for tendon repair will be of sufficient mechanical strength to support the repair site, degrade at a rate matched to tendon regeneration without causing inflammation, incorporate bioactive molecule and nanotopographic features to promote tendon regeneration. Evidently, a product which can perform in such manner will be challenging to engineer, and as further layers of complexity are added, potential complications and increasing cost would have to be considered.

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Figure 1 TERM with a focus on the musculoskeletal niche. This Venn diagram shows a selection of the range of joints currently being treated with varying elements and combinations of elements of TERM. These treatments range from the bench (IVD) via clinical trials (MSCPRPOAK) to the bedside (ACI).

Acronyms: intervertebral disc (IVD), type 1 collagen (T1Col), hyaluronan (HA), Transforming Growth FactorB1 (TGFB₁), bovine nucleus pulposus/annulus fibrosis (bNP/AF), Bone Morphogenetic Protein (BMP), Platelet-Rich Plasma (PRP), osteoarthritis (OA), Mesenchymal Stem Cells Enhanced With PRP Versus PRP In OA Knee (MSCPRPOAK).

Figure 2 Google Scholar results for articles and patents published for various musculoskeletal tissues and electrospinning over the last 15 years.

Figure 3 A schematic of a basic electrospinning circuit.

Figure 4 Diagrammatic representation of force balance occurring at the spinneret. With an increasing electrostatic force (moving from panel A-C), surface tension is overcome pulling solutions towards the collector. When these forces are balanced appropriately (C), a Taylor cone is formed and stable, continuous electrospinning is possible.

Figure 5 A summary of the search procedure to determine relevant papers. The MEDLINE/PubMed database was searched for papers published over the past five years. The paper referred to with an asterisk (*), by Hakimi *et al.* is discussed separately.

Author and date	Novel element	Materials used	Supplementary details	Outcomes
Martin JT <i>et al.</i> (2015)	Radio-opacity	PCL and radiodense zirconium (IV) oxide nanoparticles in THF and DMF	Standard electrospinning technique	<p>Cytocompatibility (with bovine BMSCs): Lower metabolic activity on scaffold vs TCP.</p> <p>Animal models: Bovine meniscal tear <i>ex vivo</i> model and murine IVD replacement <i>in vivo</i> model: scaffolds visualised by fluoroscopy and μCT.</p> <p>Histology showed biocompatibility and overlying fibrous deposition.</p> <p>Unwanted osteogenic potential: Von Kossa staining used to exclude calcium deposition on radiopaque scaffolds</p> <p>Tensile modulus: Increased up to a 25% zirconium</p>

				concentration.
Orr SB <i>et al.</i> (2015)	Multilayered scaffold	PCL dissolved at 100 mg/mL in DCM and ethanol	Procedure detail: Needle fitted with a 'round wire mesh focusing cage' of 3cm diameter and a 13.5 cm needle-to-ground distance. A glass slide was used to collect sequential layers of material, a total of 140 times.	<p>Clinically relevant size of scaffold (75mm by 100mm); 140 layers of material (1mm thick).</p> <p>Cytocompatibility (with human ASCs): Achieved complete infiltration through scaffold. Increased tenogenic gene expression (TNMD and COL3A1). Increased collagen deposition: Type I and III collagen.</p> <p>Mechanical properties: Increased yield stress and tensile modulus at day 28 vs 0.</p>
Zhao X <i>et al.</i> (2015)	Drug delivery (of MMC)	Solvent mixture of DCM and Span-80 (PLLA) was mixed with MMC/HA hydrosols, to form emulsion. DMF and PLLA were dissolved in the emulsion.	Standard electrospinning technique	<p>Cytocompatibility (with NIH/3T3 fibroblasts): Determined viability (as [MMC] increases, viability decreases), proliferation (PLLA-MMC</p>

				<p>scaffolds inhibited cell proliferation), skeletal arrangements (cell filament staining showed cytoplasmic shrinkage and reduced cell area on PLLA-MMC; flattened cell morphology of cells decreased as [MMC] increased) and apoptosis (increased apoptosis of cells increased with increasing concentration of MMC).</p> <p>Animal models: Rat achilles and rabbit FDP tendon models. Peritendinous adhesions and tendon healing evaluated by gross observation (no signs of inflammation in PLLA-MMC scaffold; severe adhesions in control, little adhesions in PLLA group), histology (PLLA-MMC had</p>
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				<p>fewest peritendinous adhesions and optimal tendon repair with increased orientation vs control), protein composition (Bax increased and Bcl-2, collagen I and III, α-SMA expressions decreased in PLLA-MMC) and inflammatory cell profile (inflammatory cells found in PLLA and least so in the controls).</p> <p>Mechanical properties: PLLA-MMC had lower maximum mechanical tensile strength <i>in vitro</i>.</p> <p>Drug release profile: Burst release profile with 26-31% released in first 48 hours and then 45-65% for subsequent 6 days.</p>
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Zhang C <i>et al.</i> (2015)	Stable jet electrospinning (SJES) – ultrafine fibres with novel materials (chitosan)	Two solutions of (1) Chitosan and gelatin in trifluoroacetic acid and (2) PLLA and PEO in dichloromethane - were mixed for the SJES substrate.	Standard electrospinning technique NB. Fibres were formed by the substrate being collected on a high-speed rotating drum.	<p>SJES produced scaffolds had improved fibre alignment.</p> <p>Cell differentiation (human iPSCs): Assessed by morphology (displayed fibroblastic phenotype vs stellate pattern in controls); gene expression (increased SCX, TNC, MKX, HoxA11, Tnmd, EphA4, Col1a1, Bgn, Fmod, Col14a1 and Dcn expression vs controls); non-osteogenic phenotype; and cell proliferation (no difference in cell proliferation vs control)</p> <p>Tensile properties: <i>in vitro</i> tensile strength six times higher and Young's modulus four times higher vs controls</p>
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				<p>Murine Achilles tendon <i>in vivo</i> model: SJES scaffold assessed by histology (improved histology scores in SJES scaffolds, increased spindle morphology and number of collagen fibres); gene expression (tenogenic Col1a1, Col5a1, Bgn, SCX, HoxA11, Tnmd, Fmod, Epha4 expression increased, osteogenic OCN and Runx2 genes down-regulated in SJES scaffold groups), ECM synthesis (increased Col I and DCN and decreased OCN in SJES group); fibre diameter of deposited collagen (collagen fibers larger by 27.8% and more numerous in SJES group vs controls) and mechanical properties (increased</p>
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				stiffness, failure force, stress at failure and Young's modulus in SJES group vs controls).
Lui YS <i>et al.</i> (2015)	Drug delivery (NPS)	<p>NPS was dissolved with blends of PLLA–PCL (stated as 90-10, 80-20).</p> <p>Scaffolds were referred to PLLA-PCL-Water%.</p>	<p>Standard electrospinning technique. NB. Fibres were collected on a collector rotating at 1500 rpm for aligned fibre morphology.</p>	<p>Beadless morphology achieved.</p> <p>Drug release profile: Increasing PLLA:PCL ratio had negative effect on speed of NPS release (average 66% release in first hour at 70:30 ratio vs 24% in 90:10).</p> <p>Adding water as co-solvent to HFIP produced sustained release of up to 3 weeks.</p> <p>Wettability significantly higher at increased PLLA:PCL ratios.</p> <p>Mechanical properties: Increased elasticity noted with decreased PLLA:PCL ratio. 90-10-3 scaffold had</p>

				<p>greatest strain at break.</p> <p>Cytocompatibility (murine fibroblasts): viability (no effect) and morphology (cells were more aligned along scaffold orientation vs controls).</p>
<p>Sheikh FA <i>et al.</i> (2015)</p>	<p>Carbothane™ 3575A nanofibers containing MWCNTs.</p>	<p>Polyurethane (Carbothane™ 3575A): produced by dissolving polymer pellets in THF and DMF.</p> <p>For nanotubes: MWCNTs in DMF added to polyurethane solutions to produce varying MWCNT concentrations (0.06%, 0.33% and 0.66%).</p>	<p>Standard electrospinning technique. NB. Rotation speeds of the collector varied from 798-5740 rpm to produce varying morphologies.</p>	<p>Defect and bead free morphology of produced scaffolds and no change in nanofibrous morphology by addition of MWCNTs.</p> <p>Fibre diameter decreased relative to increasing [MWCNT].</p> <p>MWCNTs increased porosity of nanofibres.</p> <p>Increased collector speed produced greater alignment in nanofiber structure.</p>

				<p>Cytocompatibility (with NIH/3T3 fibroblasts): viability and proliferation (exponential growth observed, scaffolds were non-toxic).</p> <p>Mechanical properties: MWCNTs in nanofibers increased tensile stress and complete alignment of nanofibers resulted in enhanced tensile stress.</p>
Zhao S <i>et al.</i> (2015)	Biodegradable gelatin-grafted PLLA fibrous membranes	bFGF and cyclodextrin in PBS were dissolved in a PLGA/chloroform solution. This solution was fabricated for electrospinning.	Standard electrospinning technique	<p>A randomly interconnected and beadless fibrous structure, with uniform fibre size was produced.</p> <p>In vitro scaffold characterisation: Determined by mechanical properties (tensile strength of PLGA and bFGF–PLGA</p>

				<p>were 3.33 ± 0.31 MPa and 3.18 ± 0.29 MPa, respectively and tensile moduli were 46.14 ± 4.42 MPa and 39.36 ± 3.69 MPa); bFGF release (ELISA found burst release of 15.4% over first 48 hours, with subsequent constant release for 14 days, and subsequent slower release for a further 8 days)</p> <p>Cytocompatibility (with HDF): Attachment and proliferation (spindle-shaped on bFGF-PLGA scaffolds; proliferation in PLGA was slower than bFGF-PLGA).</p> <p>In vivo murine model: Histology (improved collagen organization and GAG content at entheses vs controls); cell</p>
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				<p>microscopy (increased fibrocartilaginous organisation at enthesis of bFGF–PLGA scaffolds vs controls); and mechanical properties (greater ultimate load-to-failure and stiffness of supraspinatus tendon–bone construct repair bFGF-PLGA group vs controls).</p>
<p>Yang C <i>et al.</i> (2014)</p>	<p>Silk fibroin/P(LLA-CL) aligned nanoyarn-reinforced nanofibrous scaffolds (NRS)</p>	<p>Degummed silk dissolved in a ternary $\text{CaCl}_2/\text{H}_2\text{O}/\text{EtOH}$ solvent system, was dialysed against distilled water. This solution was blended with Poly(l-lactide-co-caprolactone) (P(LLA-CL)) and dissolved in HFIP</p>	<p>Procedure detail: Electrospun fibers were collected between two rotating collectors (1) a hollow metal funnel attached to a metal tube rotating at 500rpm and (2) a metal mandrel upon a linear guide slider which moved back and forth.</p>	<p>Aligned nanoyarn-reinforced nanofibrous scaffolds (NRS) had an average thickness of 600 μm.</p> <p>Scaffold morphology: NRS had greater porosity vs controls. (random and aligned nanofibrous scaffolds). More consistent fibre alignment and greater nanofiber diameters in the NRS group over</p>

				<p>controls.</p> <p>Mechanical properties of scaffolds: Parallel and perpendicular mechanical properties of scaffolds varied. NRS had higher tensile strength, Young's modulus and lower strain vs controls.</p> <p>Cytocompatibility (murine BMMSCs): Cell proliferation (NRS had higher proliferation rates vs controls); morphology (MSCs elongated on aligned nanofibrous scaffold and actin filaments paralleled axis of elongation; MSCs on NRS had inconsistent morphology with mix of elongation and random distribution between nanoyarns); infiltration</p>
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				(infiltration to 500 μm from surface of NRS vs 40 μm in random nanofibrous scaffolds and none in aligned nanofibrous scaffolds during the entire culture period).
Naghashzargar E <i>et al.</i> (2015)	Biodegradable hybrid P3HB/PCL nanofibers on a twisted SF	PCL and P3HB were separately dissolved in DMF and chloroform to produce two solvents	Procedure detail: PCL or P3HB were electrospun on surfaces of SF yarns	Cytocompatibility (with murine fibroblasts): No change in cell viability. Tensile modulus: PCL-SF had the greatest tensile load, by coating the SF yarns with electrospun nanofibers, the mechanical properties can be made more similar to those of native.
Jiang S <i>et al.</i> (2015)	Drug delivery (celecoxib)	PELA and celecoxib were dissolved in DCM and DMF	Procedure detail: Electrospinning needle was fitted with a 'round wire mesh focusing cage'	Drug release: Increasing [Celecoxib] was correlated to increasing burst release during the first 48 hours. A sustained release followed for 8 days.

				<p>Cytocompatibility (with leporine fibroblasts and tenocytes): Celecoxib was associated with decreasing cell numbers and decreased cell attachment.</p> <p>Leporine tendon model: Grossly (decreased adhesion formation noted in celecoxib scaffolds vs controls), histology (celecoxib scaffolds had decreased p-ERK1/2, p-SMAD2/3, collagen I, collagen III, COX-2 proteins in adhesion tissue).</p> <p>Mechanical properties: As concentration of celecoxib increased, maximum elongation and tensile strength decreased, and</p>
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				maximum elastic modulus increased.
He X <i>et al.</i> (2014)	Addition of nanoparticles to influence mechanical properties	Purified cotton (medical grade) was processed into both CNCs and a cellulose solution, combined and electrospun	Standard electrospinning technique to a rotating collector at different RPMs to manipulate fibre alignment	<p>Mechanical properties – CNCs at 20% w/v increased tensile strength when tested in the same direction of fibre alignment from 16.6MPa to 33.6MPa (101.7%) and elastic modulus from 0.69GPa to 1.87GPa (171.6%).</p> <p>Cytotoxicity – MTT testing assay revealed CNCs had no negative effects on cell viability and proliferation compared to pure cellulose nanofibre scaffolds</p>
Kolluru PV <i>et al.</i> (2013)	Post fabrication coating of electrospun fibres with mineral component	PLGA (MW 50-75kg/mol, 85:15 lactide/glycolide) dissolved to 25% w/v in 80/20 DCM/DMF	Standard electrospinning technique with scaffolds submerged in Simulated Body Fluid (SBF) containing 10 times more calcium and	Mechanical - PLGA fibres demonstrated uniform circular cross sections, non-uniform cross sections and uniform ellipsoidal cross section morphologies. The mechanical

			<p>phosphate than normal for variable times to induce mineralisation as thick platelet, thick conformal and thin conformal coatings</p>	<p>properties varied according to fibre cross-sectional shape.</p> <p>PLGA fibres were coated in Hydroxyapatite in thick-platelet, thick conformal and thin conformal coatings depending on submersion time in SBF. Thick platelet fibres had a higher elastic modulus of 2.49 GPa compared to 1.26GPa and 1.57GPa for thick and thin conformal coatings but a lower yield stress (29MPa) compared to thick (36MPa) and thin (48MPa) conformal coatings.</p> <p>This work has shown mineralisation of PLGA fibres is possible whilst maintaining the biological</p>
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				functionality of underlying PLGA fibres as a biomaterial.
Chainani A <i>et al.</i> (2013)	Multilayered scaffolds coated post-fabrication with tendon derived ECM (TDM), fibronectin (FN) or phosphate-buffered saline (PBS)	<p>Fresh porcine tendons, minced, lyophilized and pulverized into a powder.</p> <p>Human fibronectin</p> <p>PCL (MW 80,000) in 70/30 DCM/EtOH</p>	<p>PCL spun through conventional electrospinning techniques onto a saline bath.</p> <p>Coatings achieved through direct pipetting of solutions on to scaffolds and allowing to dry prior to UV sterilisation and use.</p> <p>hASCs seeded and cultured for up to 28 days</p>	<p>Mechanical properties – different coatings and culture times had no effect on the Young's modulus. Yield strain increased from day 0 to day 28 when seeded with hASCs. Yield stress also increased significantly over 28 days culture for FN coated scaffolds.</p> <p>Molecular – dsDNA and sulphated glycosaminoglycan levels increased over time independent of coating. Collagen content also increased, most greatly on TDM scaffolds. Collagen 1, decorin and tenascin-C (markers of tendon differentiation)</p>

				<p>increased over time independent of scaffold coating. Immunofluorescence demonstrated greater collagen 1 deposition through the full thickness of TDM scaffolds.</p> <p>Cellular – Histology showed infiltration through the entire thickness of all scaffolds.</p>
Xu <i>et al.</i> (2013)	Nanoyarn scaffold with increased porosity for cell infiltration and nutrient/waste exchange	<p>Poly(L-lactide-co-ε-caprolactone) (P(LLA-CL) (MW =300,000)</p> <p>Collagen</p> <p>Primary rabbit tenocytes</p>	<p>8% w/v 90:10 P(LLA-CL)-collagen solutions were electrospun using a modified dynamic liquid supporting system. Fibres are spun into water circulating around a vortex prior to collecting on a rotating mandrel.</p> <p>Random and Aligned</p>	<p>Morphological – Random, aligned and nanoyarn scaffolds had fibre diameters of 632nm, 643nm and 641nm respectively. These aggregated in the nanoyarn to yarn diameters of 9.51µm. The pore size of the nanoyarn (28.5µm) was much greater than the random (4.31µm) and aligned (4.43µm) scaffolds. The porosity of the random and</p>

			<p>scaffolds created through conventional methods onto a rotating collector</p>	<p>aligned scaffolds were 74.1%, 73.9% compared to 85.9% for the nanoyarn scaffold.</p> <p>Mechanical – The nanoyarn had a lower Young’s Modulus and tensile strength compared to the random and aligned nanofibrous scaffolds. After 14 days of cell culture both the tensile strength and Young’s modulus increased significantly.</p> <p>Cellular – No differences were noted in initial cell attachment but at days 4, 7 and 14 cell proliferation was significantly higher on the nanoyarn scaffold than the aligned and random nanofibrous scaffolds. Progressive cellular infiltration</p>
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				<p>into the nanoyarn scaffold was observed in contrast to the non-yarn scaffolds.</p> <p>Molecular-Nanoyarn scaffolds had higher rtPCR expression of Collagen 1, tenascin-C, biglycan and decorin compared to random nanofibrous scaffolds after 7 days of culture. These were not significantly different to aligned scaffolds. However by 14 days in culture, these genes were expressed at significantly higher levels compared to both aligned and random nanofibrous scaffolds.</p>
Liu S <i>et al.</i> (2013)	bFGF loaded nanoparticles electrospun to promote sustained	<p>Poly-l-lactic acid (PLLA), MW =50,000</p> <p>Dextran (Mw= 64,000-76,000)</p>	Dextran was mixed with bFGF, freeze dried and processed to create fine	Morphological – DGNs were observed distributed throughout electrospun fibres

	release		<p>bFGF containing dextran glassy nanoparticles (DGNs)</p> <p>bFGF DGNs were incorporated into PLLA scaffolds through both emulsion (bFGF) and direct blending (bFGF-DGNs) electrospinning onto grounded foil.</p>	<p>with surrounding areas of slightly thickened PLLA. Fibre diameters were 860nm, 810m, and 770nm respectively for PLLA, bFGF-PLLA and bFGF/DGN-PLLA scaffolds.</p> <p>Mechanical – Tensile strength of the PLLA, bFGF-PLLA and bFGF/DGN-PLLA scaffolds were 4.38MPa, 4.14MPa and 3.54MPa respectively. There was also a reduction in the maximum tensile strength for bFGF/DGN-PLLA scaffolds.</p> <p>bFGF release – bFGF/DGN-PLLA scaffolds had no burst release and sustained a release for nearly 30 days, cumulatively releasing 6400pg bFGF. bFGF-PLLA scaffolds did however exhibit a</p>
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				<p>burst release, only had a 20 day release profile with a cumulative release of only 2900.</p> <p>Cellular – In vitro - after 1 and 4 days culture, fibroblast number was significantly greater on bFGF/DGN-PLLA scaffolds compared to bFGF-PLLA and PLLA scaffolds.</p> <p>Animal model (rat) - suggestive of anti-adhesion effects of PLLA scaffolds</p>
Liu <i>et al.</i> (2012)	Bilayer hyaluronic-PCL fibrous inner layer and PCL fibrous outer layer to represent tendon sheath and reduce adhesion formation	<p>PCL (MW = 50,000)</p> <p>Hyaluronic acid</p>	<p>PCL/HA blended solutions of various ratios were prepared (0%, 4%, 8% and 12%).</p> <p>PCL and PCL/HA solutions were electrospun sequentially</p>	<p>Morphological – outer layer PCL fibres had diameters of 3.66um. Inner layers of 12%, 8%, 4% and 0% HA had diameters of 2.86um, 2.99um, 3.42um and 3.89um respectively.</p>

			<p>onto a grounded foil collector.</p> <p>Pure PCL was spun first to create outer layer followed by PCL/HA.</p>	<p>Mechanical – The tensile strength of 0%HA, 4%HA, 8%HA and 12%HA were 2.13, 1.91, 1.77 and 1.55MPa respectively. The tensile moduli were 19.82, 23.58, 26.35 and 30.13MPa respectively.</p> <p>HA release – A burst with a subsequent gradual release was seen at all HA concentrations. However the burst release percentage was lower in the 4 and 8% scaffolds, indicating a more even HA release over 15 days.</p> <p>Cellular – Cell attached and proliferation was noted to be less on the outer PCL layer compared to inner PCL/HA layers. Cell proliferation was higher as HA composition increased.</p>
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				<p>Animal models (chicken) – use of the biomimetic PCL/12%HA layer significantly reduced the severity of peritendinous adhesions and increased tendon healing scores in a chicken model compared to controls with no scaffold or with plain PCL scaffolds.</p>
<p>Ni <i>et al.</i> (2012)</p>	<p>Photochemical Tissue Bonding (PTB) of an ES silk scaffold to provide mechanical support and reduce adhesion formation</p>	<p>Silk from Bombyx Mori silkworm cocoons</p>	<p>Electrospinning was conducted on a parallel plate circuit.</p> <p>3 different methods were used to repair a rabbit Achilles tendon injury; a suture repair (SR), an PTB electrospun repair (ES/PTB) and a group combining both as a SR + ES/PTB repair.</p>	<p>Animal model (rabbit) - The use of an ES/PTB layer considerably reduced adhesions at day 7, day 14 and day 28 compared to the SR group. The use of ES/PTB alone did not provide sufficient mechanical support but the combination of a SR+ ES/PTB not only reduced adhesion but also improved mechanical properties of the healing tendon, with significantly</p>

				greater Young's modulus at day 7 and 14.
Beason <i>et al.</i> (2012)	Sacrificial fibre component (PEO) to increase scaffold porosity	Poly(e-caprolactone) (PCL) Poly(ethylene Oxide) (PEO)	<p>PCL scaffolds prepared through standardised electrospinning techniques.</p> <p>PCL/PEO scaffolds were co-electrospun. On submersion in ethanol, PEO is soluble, leaving behind a PCL scaffold with increased porosity.</p> <p>Rodent supraspinatus tear model repaired with no scaffold (control), PCL or the PCL/PEO scaffold.</p>	<p>Animal model (rat) – Cells had invaded and colonised PCL scaffolds at 4 and 8 weeks. This was surprisingly greater than the PCL/PEO scaffolds with an increased porosity. The reduction in fibre diameter may have allowed greater compression, reducing space for cell invasion and proliferation.</p>

Table 1: A summary of the review results. Acronyms:

ASCs: Adipose-derived Mesenchymal Stem Cells; BMMSCs: Bone Marrow Mesenchymal Stem Cells; CNC: Cellulose Nano-crystals; DCM: dichloromethane; DGN: dextran glassy nanoparticles; DMF: *N,N*-dimethylformamide; ECM: extracellular matrix; EtOH: ethanol; FN: fibronectin; HA: Hyaluronan;

hASC: human Adipose-derived Mesenchymal Stem Cells; HDF: Human dermal fibroblasts; HFIP: 1,1,1,3,3,3-hexafluoro-2-propanol; IVD: Intervertebral disc; MMC: mitomycin-C; MWCNTs: Multi-walled-carbon-nanotubes; NPS: Naproxen Sodium; PBS: phosphate-buffered saline; PCL: Poly(ϵ -caprolactone); PEO: poly(ethylene oxide); PLGA: Poly (lactic-co-glycolic) acid; P(LLA-CL): Poly(l-lacide-co-e-caprolactone); PLLA: poly(l-lactic acid); PTB: Photochemical Tissue Bonding; SF: Silk fibroin; SR: Suture Repair; TCP: Tissue culture plastic; THF: Tetrahydrofuran; TNMD: Tenomodulin;