Nanofibrous hydrogel composites as mechanically robust tissue engineering scaffolds

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Abstract

Hydrogels closely resemble the extracellular matrix and can support cell proliferation while new tissue is formed, making them materials of choice as tissue engineering scaffolds. However, their sometimes poor mechanical properties can hinder their application. The addition of meshes of nanofibers embedded in their matrix forms a composite that draws from the advantages of both components. As these materials are still in the early stages of development, there is a lack of uniformity across methods for characterizing their mechanical properties. A simple metric to enable comparisons between materials is proposed. The fibrous constituent improves the mechanical properties of the hydrogel, while the biocompatibility and functionality of the gels is maintained or even improved.

Keywords: hydrogel, electrospinning, mechanical testing, biocompatibility, nanofibers, composites.

Tissue Engineering

Tissue engineering is a promising treatment for severe soft and hard tissue injuries that would otherwise fail to fully recover [1, 2]. Typically, a polymeric scaffold is used to provide a framework on to which cells are seeded, allowing the cells to proliferate and develop into the functional target tissue while degrading the artificial construct. The scaffold must present biocompatibility, biodegradability, and a porous nature to allow the migration of cells and the transport of nutrients. The mechanical response of the scaffold is also of paramount importance as it must complement that of the natural tissue, particularly when this is subject to significant and complex mechanical forces, such as in the cases of bone, cartilage and skin. Also important, the physical properties of the scaffold must allow for ease of handling before and during implantation [3–6].

Hydrogels are a class of materials that meet many of these requirements. Insoluble hydrophilic polymer networks, naturally-derived or synthetic, they swell upon absorption of large amounts of water [7]. Due to their large water content, and thus close resemblance to the natural extracellular matrix (ECM), they have gained significant attention as candidates as cell scaffolds for tissue engineering applications. However, these materials are often associated with poor mechanical performance [3, 4]. For this reason, composite systems made of a hydrogel and reinforcing agents have recently gained attention. In particular, the incorporation of nanoparticulates has shown a range of improvements over hydrogels alone, reviewed in [8]. Alternatively, nanofibers have become a common addition to hydrogels for biomimetic composite construction, and such composites are the subject of this review.

Hydrogels

Interest in hydrogels for tissue engineering scaffolds arose due to their similarity to the natural ECM: hydrogels absorb large quantities of water, improving biocompatibility over bulk polymers by providing a porous environment through which cells are able to migrate and proliferate [6]. Hydrogels form through crosslinks between polymer molecules in solution, either chemically, *i.e.* by covalent bonds, or physically (Figure 1). These materials can also be loaded with bioactive agents and binding sites designed in the network structure to maintain cell viability and stimulate differentiation [9–11]. However, the presence of an interstitial fluid and its plasticizing effect degrade the mechanical response of hydrogels compared to the bulk polymer. Considerable research has therefore focused on improving the mechanical properties of hydrogels through modification of their structure.

Poly(ethylene glycol) (PEG) and poly(ethylene oxide) (PEO) are hydrophilic polymers that are extensively researched for tissue engineering applications because of their resistance to protein adsorption and consequent low immunogenicity in a physiological environment [12]. They can also be modified with acrylate or methacrylate end groups and crosslinked by exposure to light in the presence of an initiator under cytocompatible conditions [13], making them injectable, non-intrusive materials. However, these materials are well-known to be brittle and possess poor mechanical integrity when the water content is suitably large to provide for encapsulated cells [14]. Their inertness also results in very little interaction with the body.

Interpenetrating network (IPN) hydrogels are composed of two separately-crosslinked networks that share no covalent bonds. The two networks can be synthesized simultaneously or sequentially and the whole hydrogel often presents mechanical properties superior to both components [15]. A particular class of IPNs, known as double-network (DN) hydrogels, was developed with enhanced mechanical properties: the two networks are a tightly crosslinked brittle ionic polymer and a loosely crosslinked neutral polymer [16, 17]. The strength recorded for these gels is as high as tens of megapascals and they show extraordinary fracture toughness and resistance to wear, as reported in the case of acrylate-based DN gels to replicate those of natural cartilage [16, 17]. Nevertheless, the process used to form IPNs is generally not suitable for cell encapsulation [14]. Work on agarose-PEG IPNs [14, 18, 19], and other IPN systems [20, 21] all showed that this issue can be overcome but not without a detrimental effect on the mechanical properties of the material. A similar trend was reported for the incorporation of bio-ligands in IPNs to facilitate cellular adhesion and viability: recent studies have brought significant improvements in this direction, but there are still mechanical limitations [22], [23].

The physical gelation of poly(vinyl alcohol) (PVA) occurs at sub-zero temperatures [24]. Repeated cycles of freezing and thawing a solution of PVA results in the formation of crystallites that fix the polymer chains in a rigid network, known as a cryogel, with porosity between 1 and 100 μm. The technique, while not making use of potentially toxic chemical crosslinkers, also results in gels with increased strength compared to their chemically-crosslinked counterparts due to better mechanical load distribution along the network structure. Despite the promising properties of these gels, which make them

candidates for cartilage tissue engineering, PVA suffers like PEG and PEO from strong inertness in a biological environment. This prevents the material from adhering to living cells and tissues when possessing the large degrees of crosslinking required to achieve suitable stiffness [24, 25].

Nano- to micro-structured gels provide another mean of improving the mechanical response of gels. An increase in the elastic modulus of the material has been demonstrated when it was assembled from microparticles of gel molded together to form a bulk solid [26]. A similar approach, made use of gel nanoparticles crosslinked covalently in a lattice, showed a drastic increase in elasticity and toughness of the material as a result of the synergetic effect of crosslinks within and between nanogels [27]. Encapsulation of cells was not suitable and was not attempted in either of these studies.

Nanofibers

The study of nanofibers has become extensive during the past decade due to the unique properties they possess, such as very high surface to weight ratio and superior mechanical properties compared to the bulk material [28]. The great strength of nanofibers derives from highly aligned molecular chains in the structure and a low probability of surface defects, which minimizes the development of cracks [29]. They are therefore used within bioengineering for drug delivery, wound dressing and tissue engineering applications [30]. The interest in the latter is due to the similarity in morphology between a mesh of nanofibers and the collagen fibers that exist in the ECM of many tissues. Although microfibers can provide greater strength, it is preferable to use nanofibers rather than

143 microfibers for tissue engineering purposes; it has been reported that as fiber diameter
144 decreases biocompatibility increases [29], as a larger surface area is beneficial for cell
145 attachment.
146
147 New fabrication techniques are being rapidly developed that allow a wide range of
148 materials to be formed into nanofibers, particularly for tissue engineering [31]. The most
149 commonly used technique is electrospinning because of its simplicity, low cost and
150 suitability for natural and synthetic polymers, ceramics and metals [32, 33]. The process
151 works by drawing material from a blunted syringe needle using a high voltage towards an
152 earthed collecting plate, upon which a non-woven mesh of fibers is formed; the mesh can
153 be either random or aligned fibers depending upon the type of collector used. The
154 resulting fiber diameters range from a few nanometers to several micrometers [34]. There
155 are many variations of electrospinning, including using multiple needles, no needle,
156 bubble electrospinning and electroblowing, all of which can produce fibers less than 1 μm
157 in diameter [35]. Other methods capable of producing nanofibers include wetspinning
158 [36, 37], centrifugal spinning [38], microfluidic spinning, meltblowing, phase-separation
159 and drawing [35], although typically these produce fibers at the micro-scale. Coaxial
160 electrospinning is also commonly used for tissue engineering as the fibers can combine a
161 strong synthetic polymer core surrounded by a sheath of a natural polymer, such as
162 gelatin, to improve cell-fiber interactions [39].
163
164 The mechanical properties of nanofibrous meshes depend on the material properties of
165 the individual fibers, fiber diameter, mesh porosity, fiber alignment and bonding between
166 fibers. Some researchers have attempted to model how individual fibers affect the
167 mechanical properties of an electrospun mesh [40, 41], but this is yet to be fully

understood. The stiffness of individual electrospun fibers has been shown to increase with decreasing fiber diameter [42–44], however this doesn't correlate to increasing the stiffness for the overall electrospun mesh. There are multiple studies on how the solution properties, such as altering the polymer used or the polymer concentration, affect the mechanical strength of the overall mesh [45–49], however only a few have assessed how the mesh morphology affects the mechanical strength. It has been shown both that the tensile strength of the mesh increased with a decrease in fiber diameter [50] or decreased when fiber diameter decreased [51], suggesting the overall physical properties of the mesh are affected by other factors, such as pore size or the interaction between fibers. It has been theorized that for electrospun meshes it is not just fiber or mesh morphology that affects the mechanical properties, but also the conditions that were used to form the meshes [52]. The incorporation of fibrous meshes into hydrogel matrices will next be considered.

Composites

Fiber reinforced composites have been widely used throughout engineering, where the combination of two or more unlike materials can provide and allow the design of a set of properties or functions that are unattainable by any monolithic material [53]. They are particularly common in the aerospace and automotive industries due to the high strength to weight ratios the fibers can provide when combined with conventional materials [54]. Reviewed here are composites combining nano- or micro-fibers with hydrogels for tissue engineering applications, where the introduction of fibers within the gel matrix is expected to result in an improvement of the mechanical response.

192

193 *Fabrication Methods*

194

195 Methods have been suggested to combine fibers with hydrogels, including layering,
196 mixing of short fibers, and concurrent electrospinning and electrospraying [9]. The fibers
197 within composites are most commonly manufactured via electrospinning [10, 55–63].
198 Others have used fiber fragments, in the range from 1 μm to 1 mm, for applications such
199 as when the composite is to be used in minimally invasive surgeries and needs to be
200 injectable [58, 64–69]. Fibrous composites have also been made using woven microfibers
201 [37, 70, 71].

202

203 Composites containing electrospun meshes frequently use a form of wet lay-up process to
204 let the hydrogel solution infiltrate the mesh, sometimes assisted by mechanical pressure
205 [71], gentle agitation [60, 62] or vacuum assisted infiltration [69, 72]. Electrospinning has
206 also been used concurrently with electrospraying to produce a nanofibrous hydrogel
207 composite in one step [56, 59, 73]. Alternately stacking layers of fibers and hydrogel
208 forming a multilayer laminate composite is a common method [10, 57, 58, 61, 64, 70], or
209 to roll up a coated mesh to form concentric layers [55, 63, 66]. Freeze-drying is regularly
210 used to help the composite retain its shape and porosity [66, 68, 71, 74]. Nanofibre-
211 hydrogel composites have been formed from various materials by several methods and
212 can be used for any number of intended applications (Table 1). Polycaprolactone (PCL)
213 was chosen for fiber material in the majority of the studies due to its strong mechanical
214 response, FDA approved-biological inertness, and the potential to integrate biofunctional
215 motifs in its structure to influence cell behavior [57, 59, 61, 65, 66, 71–73, 75, 76].

216

Fibrous Composite Mechanics

The inclusion of fibers within hydrogels is expected to significantly improve the mechanical properties of the composite due to strain transfer between the matrix and the reinforcement [77]. There are simple bounds on the modulus of the composite E_c as a function of the volume fraction of reinforcing component V_r and the component elastic moduli, E_r for the reinforcement phase and E_g for the gel matrix. The upper bound $E_{C,U}$ corresponds to the case where fibers are aligned with the direction of loading, the lower one $E_{C,L}$ where the fibers are perpendicular (Figure 2) and are calculated as:

$$E_{C,U} = V_r E_r + (1 - V_r) E_g \quad \text{(Eq. 1)}$$

$$E_{C,L} = \left(\frac{V_r}{E_r} + \frac{(1 - V_r)}{E_g} \right)^{-1} \quad \text{(Eq. 2)}$$

Randomly aligned fibrous composites fall in the region between such two bounds. The effect of particle reinforcement is calculated according to the Hashin-Shtrikman model [78]. Fibers with some degree of alignment with the loading direction increase the stiffness of the composite significantly even at a low volume fractions.

In order to quantify the effect of reinforcement, we propose using an amplification factor, A, to facilitate comparison between studies:

$$A = \frac{E_C}{E_g} \quad \text{(Eq. 3)}$$

Combining equations 1 and 3, it is apparent that the value of A is strongly affected by the reinforcement volume fraction (V_r, Figure 2) and by the modulus mismatch between the reinforcement and gel matrix, E_r/E_g. The interaction between the fibers and the hydrogel also affects A: composites containing fibers capable of strong bonding to the hydrogel matrix will have significantly increased strain transfer, as was demonstrated by altering UHMWPE fiber surfaces to improve their interaction with a PVA hydrogel, increasing interfacial shear strength from 11 kPa to over 220 kPa [74].

Mechanical Characterization

Tensile tests are a common way to characterize fibrous composites [55, 56, 60, 62, 68–70] but unlike in traditional fields such as metallurgy, there is currently no standard methodology used across studies. Variations in test methods include: whether the composite is tested fully swollen, wet or dry, what strain rate is applied, and what geometry is used for the samples (Figure 3). Two recent studies have tensile tested nanofibrous composites based on an alginate hydrogel matrix. Tensile tests to failure were conducted on composites formed from electrospun gelatin fibers with alginate gel; the tensile elastic modulus of the alginate hydrogels alone was 77.88 ± 18.67 kPa while the inclusion of aligned gelatin fibers increased the tensile modulus to 0.50 ± 0.11 MPa (A = 6.4) [60]. The alginate failure strength was 19.29 ± 9.00 kPa, which increased to 0.34 ± 0.03 MPa with fibers, an increase 17.6 times—a version of A could equally be defined in terms of the strength or any other material property for referencing to base hydrogel properties. Similar tensile tests were conducted on composites made using electrospun PCL fibers within alginate. Depending upon the alginate concentration, the gel alone had a

267 tensile modulus ranging from 30-200 kPa, and the inclusion of randomly orientated PCL
268 fibers increased this to 180 - 400 kPa [62]. The fibers had the greatest effect in the 1%
269 alginate gel, where the modulus mismatch between gel and fibers was greatest, giving A =
270 12.7. Tensile tests performed at slow strain rates (to approximate equilibrium
271 properties) are the most direct method of evaluating the effectiveness of fiber
272 reinforcement and should thus be considered the gold standard for making comparisons
273 between studies.
274
275 Compression tests are also commonly carried out; again there is no standard methodology
276 across studies. Examples of variations include confined [71] or unconfined compression
277 tests [57][58][66][71], and creep *vs* failure tests. Two composites using chitosan hydrogel,
278 a multilayer composite using electrospun silk fibroin fibers, and one using homogeneously
279 dispersed chopped silk fibers were fabricated; the inclusion of the latter gave A = 1.9,
280 while the electrospun fibrous construct increased the stiffness of the chitosan hydrogel to
281 give A = 3.1 [58]. A composite using polyacrylamide gel and short chitosan nanofibers was
282 created, which could sustain a stress seven times higher than polyacrylamide alone at
283 95% compressive strain, and recover more of its original height [67]. This improvement
284 in mechanical properties was due to the fibers preventing the growth of microcracks and
285 the transfer of stress from the hydrogel matrix to the fibers.
286
287 Dynamic mechanical analysis (DMA) has been used to evaluate the composites, including
288 shear tests to give the complex shear modulus [64][71] and tensile tests to give the
289 storage and loss modulus for the composite [37, 59, 67, 69]. A multilayer laminate was
290 created using layers of electrospun poly(l-lactide) (PLA) fibers with a poly(lactide-co-
291 ethylene oxide fumarate) (PLEOF) hydrogel, which was tested using DMA at 37 ºC. The

292 modulus of the composite when wet was 575 ± 14 MPa, significantly greater than that for
293 the hydrogel alone (A = 4.1) and interestingly slightly greater than when the composite
294 was dry [10]. Other characterization methods have also been used, such as shear friction
295 tests [71], notched tension test [40], suture retention strength tests [55, 56], dielectric
296 property tests [37], spherical indentation [58, 62], fiber pull-out tests [74] and monotonic
297 and cyclic strain tests [63]. Regardless of test method, calculation of the amplification
298 factor, A, allows for a straightforward metric demonstrating the extent to which the
299 inclusion of reinforcement has on the mechanical properties of a hydrogel.
300
301 *Biocompatibility*
302
303 The addition of a fibrous component embedded in the hydrogel resulted in only one case
304 of lesser proliferation of cells in the studies reviewed herein: interestingly, this was when
305 natural collagen fibers were used [65]. The gel component was a hyaluronan-
306 methylcellulose blend (HAMC), which in the same study resulted in greater cell viability
307 when coupled with PCL:DLLA fibers. All other investigations reported more substantial
308 cell proliferation compared to the hydrogel alone [56– 58, 66, 71, 72, 76, 79]. This is
309 partially due to the fibers resisting the contractile forces arising from the development of
310 new tissue produced by the cells [71, 72]. PCL fibers embedded in either a cartilage-
311 derived matrix or fibrin hydrogel, both seeded with adipose-derived stem cells (ASCs),
312 were investigated, for which a chondrogenic phenotype was promoted in all cases. The
313 presence of the stronger fibrous component resulted in the geometry of the scaffold being
314 maintained during growth of the new tissue, therefore delivering a constant volume and
315 surface area to the adhered cells.
316

317 The fibers were also observed to interact directly with cells: they offer a larger number of
318 binding sites for adhesion, as explored in the case of PCL fibers and bone marrow
319 mesenchymal stem cells [66], as well as other material-cell interactions [73, 79]; they
320 provide cells with contact guidance and directionality, important for their differentiation
321 [9, 10]. The latter study, in particular, investigated the use of bone marrow stromal cells
322 seeded on composites of PLA fibers and hydroxyapatite nanocrystals embedded in a
323 poly(lactide-co-ethylene oxide fumarate) (PLEOF) gel. The addition of the fibrous
324 component resulted in greater cellular expression of osteogenic markers and more
325 pronounced cell mineralization, as a result of contact with the osteoconductive substrate.
326 Finally, the fibers can be used to fix gels to living tissues when the hydrogel component is
327 too inert to interact with the body, such as in the case of PVA [74]. Nanofibers embedded
328 in hydrogels can thus improve the biological activity within the hydrogel material and
329 improve its interaction with living tissues (Figure 4).
330

331 Concluding Remarks and Future Perspectives

332

333 Hydrogels are excellent candidate materials for tissue engineering scaffolds but they
334 generally lack sufficient mechanical performance. Borrowing a strategy from traditional
335 engineering composites, fiber-reinforced hydrogels have been developed to try and
336 overcome this natural limitation. In most cases, inclusion of fibers significantly improves
337 the mechanical properties of the hydrogel, and an amplification factor (A) has been
338 suggested as a metric for quantifying this effect. The addition of a fibrous component
339 embedded in the hydrogel not only affects the mechanical properties, but can positively
340 affect both the biocompatibility and functionality.

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References

[1] E. S. Place, N. D. Evans, and M. M. Stevens, “Complexity in biomaterials for tissue engineering.,” *Nat. Mater.*, vol. 8, no. 6, pp. 457–70, Jun. 2009.

[2] H. Naderi, M. M. Matin, and A. R. Bahrami, “Review paper: critical issues in tissue engineering: biomaterials, cell sources, angiogenesis, and drug delivery systems.,” *J. Biomater. Appl.*, vol. 26, no. 4, pp. 383–417, Nov. 2011.

[3] S. D. McCullen, C. M. Haslauer, and E. G. Loboa, “Fiber-reinforced scaffolds for tissue engineering and regenerative medicine: use of traditional textile substrates to nanofibrous arrays,” *J. Mater. Chem.*, vol. 20, no. 40, p. 8776, Oct. 2010.

[4] X. Li, Y. Yang, Y. Fan, Q. Feng, F. Cui, and F. Watari, “Biocomposites reinforced by fibers or tubes as scaffolds for tissue engineering or regenerative medicine.,” *J. Biomed. Mater. Res. A*, vol. 102, no. 5, pp. 1580–94, May 2014.

[5] Y.-K. Seo and J.-K. Park, “Tissue engineered scaffold utilizing the reinforced technique,” *Biotechnol. Bioprocess Eng.*, vol. 15, no. 4, pp. 527–533, Sep. 2010.

[6] J. M. Shapiro and M. L. Oyen, “Hydrogel Composite Materials for Tissue Engineering Scaffolds,” *JOM*, vol. 65, no. 4, pp. 505–516, Mar. 2013.

[7] N. Annabi, A. Tamayol, J. A. Uquillas, M. Akbari, L. E. Bertassoni, C. Cha, G. Camci-Unal, M. R. Dokmeci, N. a Peppas, and A. Khademhosseini, “25th Anniversary Article: Rational Design and Applications of Hydrogels in Regenerative Medicine.,” *Adv. Mater. Res.*, vol. 26, no. 1, pp. 85–124, Jan. 2014.

[8] A. K. Gaharwar, N. A. Peppas, and A. Khademhosseini, “Nanocomposite hydrogels for biomedical applications.,” *Biotechnol. Bioeng.*, vol. 111, no. 3, pp. 441–53, Mar. 2014.

[9] L. A. Bosworth, L.-A. Turner, and S. H. Cartmell, “State of the art composites comprising electrospun fibres coupled with hydrogels: a review,” *Nanomedicine Nanotechnology, Biol. Med.*, vol. 9, no. 3, pp. 322–335, 2013.

[10] W. Xu, J. Ma, and E. Jabbari, “Material properties and osteogenic differentiation of marrow stromal cells on fiber-reinforced laminated hydrogel nanocomposites,” *Acta Biomater.*, vol. 6, no. 6, pp. 1992–2002, 2010.

[11] M. W. Tibbitt and K. S. Anseth, “Hydrogels as extracellular matrix mimics for 3D cell culture.,” *Biotechnol. Bioeng.*, vol. 103, no. 4, pp. 655–63, Jul. 2009.

[12] A. M. Kloxin, C. J. Kloxin, C. N. Bowman, and K. S. Anseth, “Mechanical properties of cellularly responsive hydrogels and their experimental determination.,” *Adv. Mater.*, vol. 22, no. 31, pp. 3484–94, Aug. 2010.

[13] J. L. Drury and D. J. Mooney, “Hydrogels for tissue engineering: scaffold design variables and applications,” *Biomaterials*, vol. 24, no. 24, pp. 4337–4351, Nov. 2003.

[14] B. J. DeKosky, N. H. Dormer, G. C. Ingavle, C. H. Roatch, J. Lomakin, M. S. Detamore, and S. H. Gehrke, “Hierarchically designed agarose and poly(ethylene glycol) interpenetrating network hydrogels for cartilage tissue engineering.,” *Tissue Eng. Part C. Methods*, vol. 16, no. 6, pp. 1533–42, Dec. 2010.

[15] E. S. Dragan, “Design and applications of interpenetrating polymer network hydrogels. A review,” *Chem. Eng. J.*, vol. 243, pp. 572–590, May 2014.

[16] M. A. Haque, T. Kurokawa, and J. P. Gong, “Super tough double network hydrogels and their application as biomaterials,” *Polymer (Guildf).*, vol. 53, no. 9, pp. 1805–1822, Apr. 2012.

[17] S. Ronken, D. Wirz, a U. Daniels, T. Kurokawa, J. P. Gong, and M. P. Arnold, “Double-network acrylamide hydrogel compositions adapted to achieve cartilage-like dynamic stiffness.,” *Biomech. Model. Mechanobiol.*, vol. 12, no. 2, pp. 243–8, Apr. 2013.

[18] D. a Rennerfeldt, A. N. Renth, Z. Talata, S. H. Gehrke, and M. S. Detamore, “Tuning mechanical performance of poly(ethylene glycol) and agarose interpenetrating network hydrogels for cartilage tissue engineering.,” *Biomaterials*, vol. 34, no. 33, pp. 8241–57, Nov. 2013.

[19] G. C. Ingavle, N. H. Dormer, S. H. Gehrke, and M. S. Detamore, “Using chondroitin sulfate to improve the viability and biosynthesis of chondrocytes encapsulated in interpenetrating network (IPN) hydrogels of agarose and poly(ethylene glycol) diacrylate.,” *J. Mater. Sci. Mater. Med.*, vol. 23, no. 1, pp. 157–70, Jan. 2012.

[20] H. Shin, B. D. Olsen, and A. Khademhosseini, “The mechanical properties and cytotoxicity of cell-laden double-network hydrogels based on photocrosslinkable gelatin and gellan gum biomacromolecules.,” *Biomaterials*, vol. 33, no. 11, pp. 3143–52, Apr. 2012.

[21] M. a Daniele, A. a Adams, J. Naciri, S. H. North, and F. S. Ligler, “Interpenetrating networks based on gelatin methacrylamide and PEG formed using concurrent thiol click chemistries for hydrogel tissue engineering scaffolds.,” *Biomaterials*, vol. 35, no. 6, pp. 1845–56, Feb. 2014.

[22] X. Tong and F. Yang, “Engineering interpenetrating network hydrogels as biomimetic cell niche with independently tunable biochemical and mechanical properties.,” *Biomaterials*, vol. 35, no. 6, pp. 1807–15, Feb. 2014.

[23] Y. Zhao, T. Nakajima, J. J. Yang, T. Kurokawa, J. Liu, J. Lu, S. Mizumoto, K. Sugahara, N. Kitamura, K. Yasuda, a U. D. Daniels, and J. P. Gong, “Proteoglycans and glycosaminoglycans improve toughness of biocompatible double network hydrogels.,” *Adv. Mater.*, vol. 26, no. 3, pp. 436–42, Jan. 2014.

[24] C. M. Hassan and N. A. Peppas, “Structure and Applications of Poly (vinyl alcohol) Hydrogels Produced by Conventional Crosslinking or by Freezing / Thawing Methods,” *Adv. Polym. Sci.*, vol. 153, pp. 37–65, 2000.

[25] M. Kobayashi and H. S. Hyu, “Development and Evaluation of Polyvinyl Alcohol-Hydrogels as an Artificial Atrticular Cartilage for Orthopedic Implants,” *Materials (Basel).*, vol. 3, no. 4, pp. 2753–2771, Apr. 2010.

[26] S. Park, S. Hwang, and J. Lee, “pH-responsive hydrogels from moldable composite microparticles prepared by coaxial electro-spray drying,” *Chem. Eng. J.*, vol. 169, no. 1–3, pp. 348–357, May 2011.

[27] L.-W. Xia, R. Xie, X.-J. Ju, W. Wang, Q. Chen, and L.-Y. Chu, “Nano-structured smart hydrogels with rapid response and high elasticity,” *Nat. Commun.*, vol. 4, 2013.

[28] H. S. Yoo, T. G. Kim, and T. G. Park, “Surface-functionalized electrospun nanofibers for tissue engineering and drug delivery.,” *Adv. Drug Deliv. Rev.*, vol. 61, no. 12, pp. 1033–42, Oct. 2009.

[29] U. Boudriot, R. Dersch, A. Greiner, and J. H. Wendorff, “Electrospinning approaches toward scaffold engineering--a brief overview.,” *Artif. Organs*, vol. 30, no. 10, pp. 785–92, Oct. 2006.

[30] S. Ramakrishna, K. Fujihara, W.-E. Teo, T.-C. Lim, and Z. Ma, *An Introduction to Electrospinning and Nanofibres*. Singapore: World Scientific Publishing, 2005, pp. 275–339.

[31] A. Tamayol, M. Akbari, N. Annabi, A. Paul, A. Khademhosseini, and D. Juncker, “Fiber-based tissue engineering: Progress, challenges, and opportunities.,” *Biotechnol. Adv.*, vol. 31, no. 5, pp. 669–87, Jan. 2013.

[32] S. Agarwal, A. Greiner, and J. H. Wendorff, “Functional materials by electrospinning of polymers,” *Prog. Polym. Sci.*, vol. 38, no. 6, pp. 963–991, Jun. 2013.

[33] N. Bhardwaj and S. C. Kundu, “Electrospinning: a fascinating fiber fabrication technique.,” *Biotechnol. Adv.*, vol. 28, no. 3, pp. 325–47, Jan. 2010.

[34] A. Rogina, “Electrospinning process: Versatile preparation method for biodegradable and natural polymers and biocomposite systems applied in tissue engineering and drug delivery,” *Appl. Surf. Sci.*, vol. 296, pp. 221–230, Mar. 2014.

[35] R. Nayak, R. Padhye, I. L. Kyratzis, Y. B. Truong, and L. Arnold, “Recent advances in nanofibre fabrication techniques,” *Text. Res. J.*, vol. 82, no. 2, pp. 129–147, Oct. 2011.

[36] S. Iwamoto, A. Isogai, and T. Iwata, “Structure and mechanical properties of wet-spun fibers made from natural cellulose nanofibers.,” *Biomacromolecules*, vol. 12, no. 3, pp. 831–6, Mar. 2011.

[37] L. Liu, B. Wang, Y. Gao, and T.-C. Bai, “Chitosan fibers enhanced gellan gum hydrogels with superior mechanical properties and water-holding capacity.,” *Carbohydr. Polym.*, vol. 97, no. 1, pp. 152–8, Aug. 2013.

[38] L. Ren, V. Pandit, J. Elkin, T. Denman, J. A. Cooper, and S. P. Kotha, “Large-scale and highly efficient synthesis of micro- and nano-fibers with controlled fiber morphology by centrifugal jet spinning for tissue regeneration.,” *Nanoscale*, vol. 5, no. 6, pp. 2337–45, Mar. 2013.

[39] V. M. Merkle, L. Zeng, M. J. Slepian, and X. Wu, “Core-shell nanofibers: Integrating the bioactivity of gelatin and the mechanical property of polyvinyl alcohol.,” *Biopolymers*, vol. 101, no. 4, pp. 336–46, Apr. 2014.

[40] C. . Koh, D. G. T. Strange, K. Tonsomboon, and M. L. Oyen, “Failure mechanisms in fibrous scaffolds.,” *Acta Biomater.*, vol. 9, no. 7, pp. 7326–34, Jul. 2013.

[41] A. Camposeo, I. Greenfeld, F. Tantussi, S. Pagliara, M. Moffa, F. Fuso, M. Allegrini, E. Zussman, and D. Pisignano, “Local mechanical properties of electrospun fibers correlate to their internal nanostructure.,” *Nano Lett.*, vol. 13, no. 11, pp. 5056–62, Nov. 2013.

[42] M. K. Shin, S. I. Kim, S. J. Kim, S.-K. Kim, H. Lee, and G. M. Spinks, “Size-dependent elastic modulus of single electroactive polymer nanofibers,” *Appl. Phys. Lett.*, vol. 89, no. 23, p. 231929, Dec. 2006.

[43] S.-C. Wong, A. Baji, and S. Leng, “Effect of fiber diameter on tensile properties of electrospun poly(ε-caprolactone),” *Polymer (Guildf).*, vol. 49, no. 21, pp. 4713–4722, Oct. 2008.

[44] B. Janković, J. Pelipenko, M. Škarabot, I. Muševič, and J. Kristl, “The design trend in tissue-engineering scaffolds based on nanomechanical properties of individual electrospun nanofibers.,” *Int. J. Pharm.*, vol. 455, no. 1–2, pp. 338–47, Oct. 2013.

[45] A. Yin, K. Zhang, M. J. McClure, C. Huang, J. Wu, J. Fang, X. Mo, G. L. Bowlin, S. S. Al-Deyab, and M. El-Newehy, “Electrospinning collagen/chitosan/poly(L-lactic acid-co-ε-caprolactone) to form a vascular graft: mechanical and biological characterization.,” *J. Biomed. Mater. Res. A*, vol. 101, no. 5, pp. 1292–301, May 2013.

[46] R. Machado, A. da Costa, V. Sencadas, C. Garcia-Arévalo, C. M. Costa, J. Padrão, A. Gomes, S. Lanceros-Méndez, J. C. Rodríguez-Cabello, and M. Casal, “Electrospun silk-elastin-like fibre mats for tissue engineering applications.,” *Biomed. Mater.*, vol. 8, no. 6, p. 065009, Dec. 2013.

[47] T. Nuge, M. E. Hoque, T. K. Yeow, N. Nordin, and M. Chowdhury, “Electrospun gelatin composite nanofibres: A review on structural and mechanical characterizations,” *Regenerative Research*, 2013. [Online]. Available: http://www.regres.tesma.org.my/pdf/RR-271113-009 R1 (7) final.pdf. [Accessed: 03-Mar-2014].

[48] N. Kimura, B.-S. Kim, and I.-S. Kim, “Effects of Fe 2+ ions on morphologies, microstructures and mechanical properties of electrospun nylon-6 nanofibers,” *Polym. Int.*, vol. 63, no. 2, pp. 266–272, Feb. 2014.

[49] Y. Ji, K. Liang, X. Shen, and G. L. Bowlin, “Electrospinning and characterization of chitin nanofibril/polycaprolactone nanocomposite fiber mats.,” *Carbohydr. Polym.*, vol. 101, pp. 68–74, Jan. 2014.

[50] N. T. B. Linh, Y. K. Min, H.-Y. Song, and B.-T. Lee, “Fabrication of polyvinyl alcohol/gelatin nanofiber composites and evaluation of their material properties.,” *J. Biomed. Mater. Res. B. Appl. Biomater.*, vol. 95, no. 1, pp. 184–91, Oct. 2010.

[51] M. C. McManus, E. D. Boland, H. P. Koo, C. P. Barnes, K. J. Pawlowski, G. E. Wnek, D. G. Simpson, and G. L. Bowlin, “Mechanical properties of electrospun fibrinogen structures.,” *Acta Biomater.*, vol. 2, no. 1, pp. 19–28, Jan. 2006.

[52] M. Richard-Lacroix and C. Pellerin, “Molecular Orientation in Electrospun Fibers: From Mats to Single Fibers,” *Macromolecules*, vol. 46, no. 24, pp. 9473–9493, Dec. 2013.

[53] R. M. Christensen, *Mechanics of Composite Materials*. New York, USA: Dover Publications, 2005.

[54] C. Soutis, “Fibre reinforced composites in aircraft construction,” *Prog. Aerosp. Sci.*, vol. 41, no. 2, pp. 143–151, Feb. 2005.

[55] R. E. McMahon, X. Qu, A. C. Jimenez-Vergara, C. A. Bashur, S. A. Guelcher, A. S. Goldstein, and M. S. Hahn, “Hydrogel-electrospun mesh composites for coronary artery bypass grafts.,” *Tissue Eng. Part C. Methods*, vol. 17, no. 4, pp. 451–61, Apr. 2011.

[56] Y. Hong, A. Huber, K. Takanari, N. J. Amoroso, R. Hashizume, S. F. Badylak, and W. R. Wagner, “Mechanical properties and in vivo behavior of a biodegradable synthetic polymer microfiber-extracellular matrix hydrogel biohybrid scaffold.,” *Biomaterials*, vol. 32, no. 13, pp. 3387–94, May 2011.

[57] J. Coburn, M. Gibson, and P. Bandalini, “Biomimetics of the extracellular matrix: an integrated three-dimensional fiber-hydrogel composite for cartilage tissue engineering,” *Smart Struct. Syst.*, vol. 7, no. 3, pp. 213–222, 2011.

[58] F. Mirahmadi, M. Tafazzoli-Shadpour, M. A. Shokrgozar, and S. Bonakdar, “Enhanced mechanical properties of thermosensitive chitosan hydrogel by silk fibers for cartilage tissue engineering,” *Mater. Sci. Eng. C*, vol. 33, no. 8, pp. 4786–4794, 2013.

[59] A. Thorvaldsson, J. Silva-Correia, J. M. Oliveira, R. L. Reis, P. Gatenholm, and P. Walkenström, “Development of nanofiber-reinforced hydrogel scaffolds for nucleus pulposus regeneration by a combination of electrospinning and spraying technique,” *J. Appl. Polym. Sci.*, vol. 128, no. 2, pp. 1158–1163, Apr. 2013.

[60] K. Tonsomboon and M. L. Oyen, “Composite electrospun gelatin fiber-alginate gel scaffolds for mechanically robust tissue engineered cornea.,” *J. Mech. Behav. Biomed. Mater.*, vol. 21, pp. 185–94, May 2013.

[61] N. Han, P. A. Bradley, J. Johnson, K. S. Parikh, A. Hissong, M. A. Calhoun, J. J. Lannutti, and J. O. Winter, “Effects of hydrophobicity and mat thickness on release from hydrogel-electrospun fiber mat composites.,” *J. Biomater. Sci. Polym. Ed.*, vol. 24, no. 17, pp. 2018–30, Jan. 2013.

[62] D. G. T. Strange, K. Tonsomboon, and M. L. Oyen, “Mechanical behaviour of electrospun fibre-reinforced hydrogels.,” *J. Mater. Sci. Mater. Med.*, Jan. 2014.

[63] P. S. Thayer, A. F. Dimling, D. S. Plessl, M. R. Hahn, S. A. Guelcher, L. A. Dahlgren, and A. S. Goldstein, “Cellularized cylindrical fiber/hydrogel composites for ligament tissue engineering.,” *Biomacromolecules*, vol. 15, no. 1, pp. 75–83, Jan. 2014.

[64] O. Regev, C. S. Reddy, N. Nseir, and E. Zussman, “Hydrogel Reinforced by Short Albumin Fibers: Mechanical Characterization and Assessment of Biocompatibility,” *Macromol. Mater. Eng.*, vol. 298, no. 3, pp. 283–291, Mar. 2013.

[65] A. Hsieh, T. Zahir, Y. Lapitsky, B. Amsden, W. Wan, and M. S. Shoichet, “Hydrogel/electrospun fiber composites influence neural stem/progenitor cell fate,” *Soft Matter*, vol. 6, no. 10, p. 2227, May 2010.

[66] D. Kai, M. P. Prabhakaran, B. Stahl, M. Eblenkamp, E. Wintermantel, and S. Ramakrishna, “Mechanical properties and in vitro behavior of nanofiber-hydrogel composites for tissue engineering applications.,” *Nanotechnology*, vol. 23, no. 9, p. 095705, Mar. 2012.

[67] C. Zhou and Q. Wu, “A novel polyacrylamide nanocomposite hydrogel reinforced with natural chitosan nanofibers.,” *Colloids Surf. B. Biointerfaces*, vol. 84, no. 1, pp. 155–62, May 2011.

[68] J. S. Gonzalez, L. N. Ludueña, A. Ponce, and V. A. Alvarez, “Poly(vinyl alcohol)/cellulose nanowhiskers nanocomposite hydrogels for potential wound dressings.,” *Mater. Sci. Eng. C. Mater. Biol. Appl.*, vol. 34, pp. 54–61, Jan. 2014.

[69] M. Jonoobi, Y. Aitomäki, A. P. Mathew, and K. Oksman, “Thermoplastic polymer impregnation of cellulose nanofibre networks: Morphology, mechanical and optical properties,” *Compos. Part A Appl. Sci. Manuf.*, vol. 58, pp. 30–35, Mar. 2014.

[70] J. L. Holloway, A. M. Lowman, and G. R. Palmese, “Mechanical evaluation of poly(vinyl alcohol)-based fibrous composites as biomaterials for meniscal tissue replacement.,” *Acta Biomater.*, vol. 6, no. 12, pp. 4716–24, Dec. 2010.

[71] F. T. Moutos, B. T. Estes, and F. Guilak, “Multifunctional hybrid three-dimensionally woven scaffolds for cartilage tissue engineering.,” *Macromol. Biosci.*, vol. 10, no. 11, pp. 1355–64, Nov. 2010.

[72] F. T. Moutos and F. Guilak, “Functional properties of cell-seeded three-dimensionally woven poly(epsilon-caprolactone) scaffolds for cartilage tissue engineering.,” *Tissue Eng. Part A*, vol. 16, no. 4, pp. 1291–301, Apr. 2010.

[73] A. K. Ekaputra, G. D. Prestwich, S. M. Cool, and D. W. Hutmacher, “Combining electrospun scaffolds with electrosprayed hydrogels leads to three-dimensional cellularization of hybrid constructs.,” *Biomacromolecules*, vol. 9, no. 8, pp. 2097–103, Aug. 2008.

[74] J. L. Holloway, A. M. Lowman, M. R. VanLandingham, and G. R. Palmese, “Chemical grafting for improved interfacial shear strength in UHMWPE/PVA-hydrogel fiber-based composites used as soft fibrous tissue replacements,” *Compos. Sci. Technol.*, vol. 85, pp. 118–125, Aug. 2013.

[75] I.-C. Liao, F. T. Moutos, B. T. Estes, X. Zhao, and F. Guilak, “Composite Three-Dimensional Woven Scaffolds with Interpenetrating Network Hydrogels to Create Functional Synthetic Articular Cartilage,” *Adv. Funct. Mater.*, vol. 23, no. 47, pp. 5833–5839, Dec. 2013.

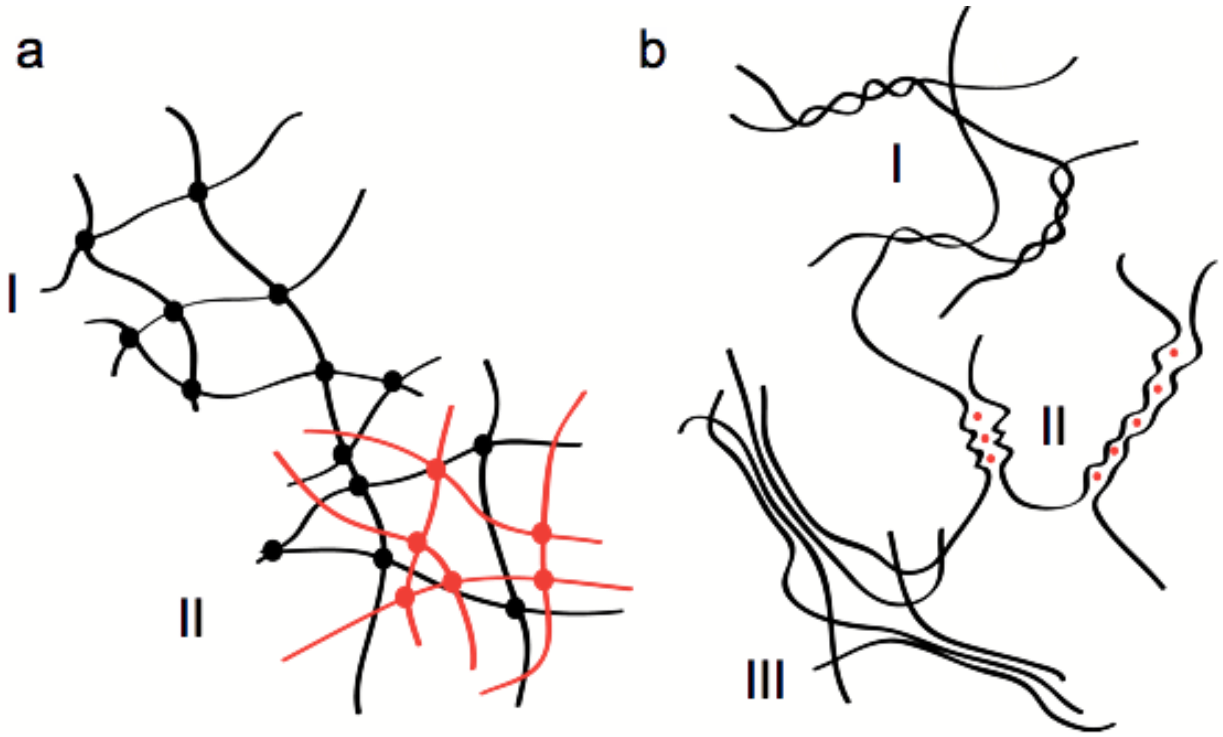
[76] W. Liu, J. Zhan, Y. Su, T. Wu, S. Ramakrishna, S. Liao, and X. Mo, “Injectable hydrogel incorporating with nanoyarn for bone regeneration.,” *J. Biomater. Sci. Polym. Ed.*, vol. 25, no. 2, pp. 168–80, Jan. 2014.

[77] K. K. Chawla, *Composite Materials: Science and Engineering*. Springer Science+Business Media, 1987.

[78] W. D. Kingery, H. K. Bowen, and D. R. Uhlmann, *Introduction to Ceramics*. Wiley-Blackwell, 1976.

[79] S. Sakai, Y. Takagi, Y. Yamada, T. Yamaguchi, and K. Kawakami, “Reinforcement of porous alginate scaffolds by incorporating electrospun fibres.,” *Biomed. Mater.*, vol. 3, no. 3, p. 034102, Sep. 2008.

588 **Figure Captions**



589

590 Figure 1. Methods of formation of polymer network structures. (a) I. Chemical crosslinks;
591 II. IPN showing two covalently crosslinked hydrogels. (b) Examples of physical crosslinks:
592 I. Steric hindrance by chain coiling between long chains in carrageenan; II. Electrostatic
593 attraction to Ca^{2+} ions in alginate hydrogels; III. Formation of crystallites in PVA
594 hydrogels.

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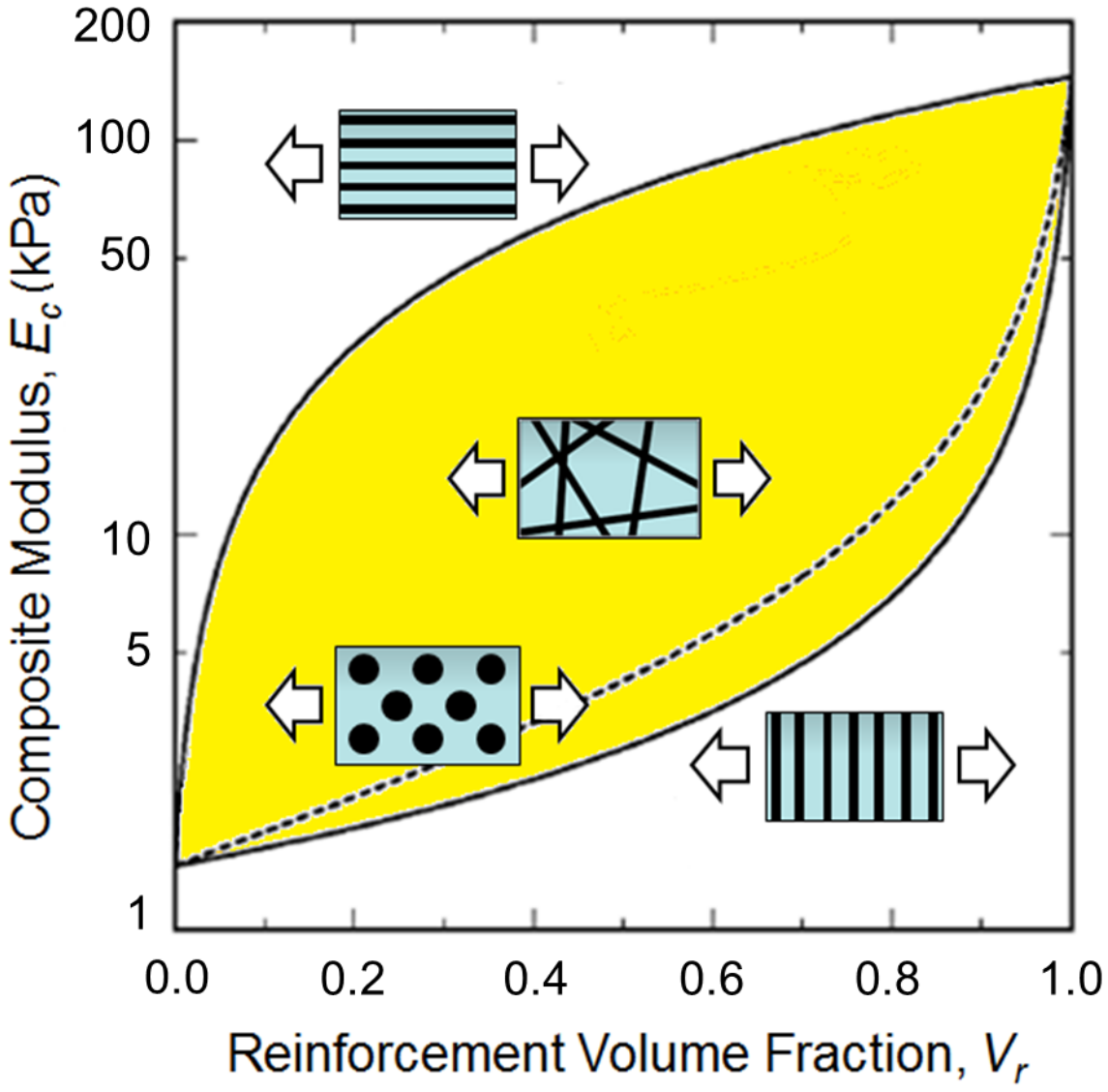

596

597 Figure 2. Schematic illustration of the composite modulus as a function of volume fraction

598 and orientation of fibers (shaded area) and particles (dashed line).

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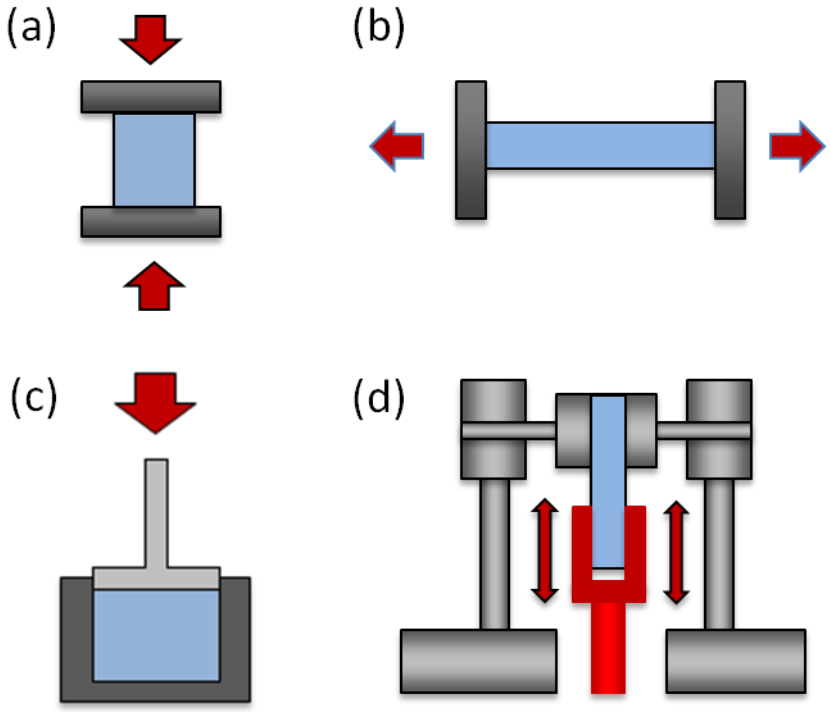

600

601 Figure 3. Schematics of mechanical tests for fiber-reinforced composites: (a) compression,

602 (b) tension, (c) confined compression, and (d) dynamic testing.

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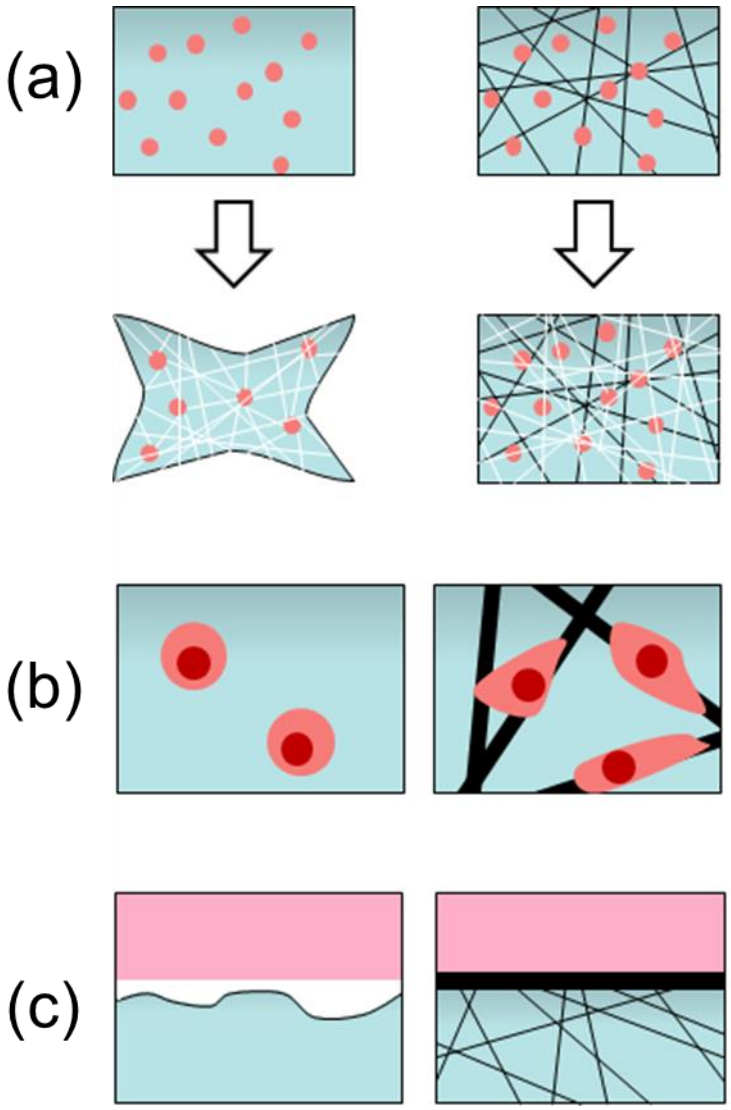

Figure 4. Nanofibers embedded in hydrogels provide enhanced biological activity by (a) resistance to contraction during the development of new tissue, (b) provision of attachment sites and contact directionality to cells, (c) improved binding to body tissues.

Table 1: Materials and methods used to make nanofibrous hydrogel composites with the intended application

Fiber	Matrix	Manufacture method	Application	Refs.
Polypropylene - Melt-blown microfibers	PVA	Infiltration, multilayer laminate, freeze-dry	Cartilage - meniscus	[70]
UHMWPE[a] - woven				

microfibers				
UHMWPE - woven microfibers	PVA	PVA grafted to fiber surface	Cartilage - meniscus	[74]
PCL - 3D woven microfibers	Porcine-derived cartilage	Infiltration, freeze-dry	Cartilage - articular	[71]
PCL - 3D woven microfibers	Fibrin gel	Vacuum-assisted infiltration	Cartilage - articular	[72]
PCL - electrospun	Poly(ethylene glycol)-diacrylate (PEGDA)	Infiltration, multilayer laminate	Cartilage - unspecified	[57]
PCL - electrospun	PEG- poly(lactic acid) (PEGPLA)	Infiltration, multilayer laminate	Controlled drug release systems	[61]
PCL - electrospun	Alginate	Infiltration	Cartilage - intervertebral disc	[62]
PCL - electrospun	Gellan gum	Concurrent electrospinning/ electrospraying	Cartilage - intervertebral disc	[59]
PCL/gelatin - blend & coaxial electrospun	Gelatin	Dispersed in hydrogel, rolled laminate, freeze-dry	ECM - Unspecified	[66]
PCL with DLLA - electrospun Collagen - electrospun fiber fragments	Hyaluronan & methylcellulose	Dispersed fragments in hydrogel	Spinal tissue	[65]
PEUUR[b] - electrospun	PEG-fibrin	Infiltration, rolled laminate	Coronary artery bypass grafts	[55]
PEUUR - electrospun	Porcine dermal ECM	Concurrent electrospinning/ electrospraying	Unspecified soft tissues	[56]
PEUUR - electrospun PLGA - electrospun	PEG	Infiltration, rolled laminate	Ligament	[63]
PLA - electrospun	Poly(lactide-co-ethylene	Infiltration, multilayer	Bone	[10]

	oxide fumarate)	laminate		
Chitosan - chopped nanofibers	Polyacrylamide	Dispersed in hydrogel	Unspecified	[67]
Chitosan - wetspun, chopped microfibers	Gellan gum	Dispersed in hydrogel	Unspecified	[37]
Silk fibroin - electrospun	Chitosan/ glycerophosphate	Infiltration, multilayer laminate, sol–gel transition	Cartilage - articular	[58]
Degummed silk fibers - chopped		Dispersed in hydrogel, sol–gel transition		
Serum albumin-derived - short electrospun	Gelatin	Infiltration, multilayer laminate	Unspecified	[64]
Gelatin - electrospun	Alginate	Infiltration	Cornea	[60]
Cellulose nanowhiskers	Polyvinyl alcohol	Dispersed in hydrogel, freeze-thawed	Wound dressing	[68]
Cellulose nanofibers	Cellulose acetate butyrate	Vacuum assisted infiltration, compression molded	Unspecified	[69]

611

612 [a] Ultra-High Molecular Weight Polyethylene

613 [b] Polyester urethane urea