Review

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Nanofibrous hydrogel composites as mechanically robust tissue engineering scaffolds

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Hydrogels closely resemble the extracellular matrix (ECM) 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. Given that these materials are still in the early stages of development, there is a lack of uniformity across methods for characterizing their mechanical properties. Here, we propose a simple metric to enable comparisons between materials. The fibrous constituent improves the mechanical properties of the hydrogel, while the biocompatibility and functionality of the gels are maintained or even improved.

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 and biodegradability, and be porous in nature to allow the migration of cells and the transport of nutrients. The mechanical response of the scaffold is also important because 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. These are insoluble hydrophilic polymer networks, either naturally derived or synthetic, that swell upon absorption of large amounts of water [7]. Given their large water content and, thus, close resemblance to the natural ECM, they have gained significant attention as candidates for cell scaffolds for tissue engineering applications. However, these materials are often associated with poor mechanical performance [3,4]. For this reason, composite systems comprising 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, that is, 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 with the bulk polymer. Therefore, considerable research has 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, nonintrusive materials. However, these materials are well known to be brittle and have poor mechanical integrity when the water content is suitably large to provide for encapsulated cells [14]. Their inertness also results in little interaction with the body.

Interpenetrating network (IPN) hydrogels comprise 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 that are superior to both components

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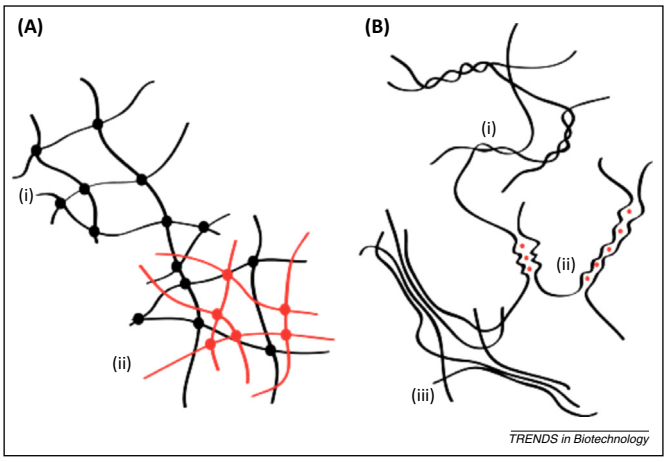

Figure 1. Methods of formation of polymer network structures. **(A)** (i) Chemical crosslinks; (ii) Interpenetrating network showing two covalently crosslinked hydrogels. **(B)** Examples of physical crosslinks: (i) steric hindrance by chain coiling between long chains in carrageenan; (ii) electrostatic attraction to Ca^{2+} ions in alginate hydrogels; and (iii) formation of crystallites in poly(vinyl alcohol) hydrogels.

[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] 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 bioligands 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 with 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, similar to PEG and PEO, from strong inertness in a biological environment. This prevents the material from adhering to living cells and tissues when having the large degrees of crosslinking required to achieve suitable stiffness [24,25].

Nano- to microstructured gels provide another means 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, and showed an 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 their unique properties, such as very high surface to weight ratio, and superior mechanical properties compared with 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]. Therefore, they are 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 microfibers for tissue engineering purposes; it has been reported that, because as fiber diameter decreases biocompatibility increases [29], a larger surface area is beneficial for cell attachment.

New fabrication techniques are being rapidly developed that allow a range of materials to be formed into nanofibers, particularly for tissue engineering [31]. The most commonly used technique is electrospinning because of its simplicity, low cost, and suitability for natural and synthetic polymers, ceramics, and metals [32,33]. The process works by drawing material from a blunted syringe needle using a high voltage towards an earthed collecting plate, upon which a nonwoven mesh of fibers is formed; the mesh can be either random or aligned fibers depending upon the type of collector used. The resulting fiber diameters range from a few nanometers to several micrometers [34]. There are many variations of electrospinning, including using multiple needles, no needle, bubble electrospinning, and electroblowing, all of which can produce fibers less than 1 μm in diameter [35]. Other methods capable of producing nanofibers include wetspinning [36,37], centrifugal spinning [38], microfluidic spinning, meltblowing, phase-separation, and drawing [35], although typically these produce fibers at the microscale. Coaxial electrospinning is also commonly used for tissue engineering because the fibers can combine a strong synthetic polymer core surrounded by a sheath of a natural polymer, such as gelatin, to improve cell–fiber interactions [39].

The mechanical properties of nanofibrous meshes depend on the material properties of the individual fibers, fiber diameter, mesh porosity, fiber alignment, and bonding between fibers. Some researchers have attempted to model how individual fibers affect the 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 does not correlate with increasing the stiffness for the overall electrospun mesh. There are