An Integrated Design, Material, and Fabrication Platform for Engineering Biomechanically and Biologically Functional Soft Tissues

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ABSTRACT: We present a design rationale for stretchable soft network composites for engineering tissues that predominantly function under high tensile loads. The convergence of 3D-printed fibers selected from a design library and biodegradable interpenetrating polymer networks (IPNs) result in biomimetic tissue engineered constructs (bTECs) with fully tunable properties that can match specific tissue requirements. We present our technology platform using an exemplary soft network composite model that is characterized to be flexible, yet ∼125 times stronger (E = 3.19 MPa) and ∼100 times tougher (W⊥ = ∼2000 kJ m−3) than its hydrogel counterpart.

KEYWORDS: biomimetic, soft network composite, tissue engineering, hydrogel, 3D printing, fiber reinforcement, melt electrospinning writing, interpenetrating polymer network

Although engineers and scientists mainly embark on a quest for stronger, harder, and tougher materials, there is an increasing need for advanced soft materials that are mechanically functional and highly compliant with the human body. A huge potential for soft materials exhibiting good biocompatibility, shape adaptability, and in particular, mechanical performance comparable to those of their biological counterparts can be found in emerging applications and fields including soft-bodied robotics,1 stretchable electronics and medical devices,2,3 and tissue engineering and regenerative medicine (TE&RM).4 However, soft biological materials such as skin, tendon, ligament, cartilage, heart, muscle, etc. are often highly flexible yet able to absorb high stresses without failure while exhibiting complex biomechanical behaviors (e.g., anisotropy, nonlinearity and viscoelasticity) which make them difficult to engineer. Above all, in the case of TE&RM, biologically functional biomaterials capable of inducing and regulating tissue regeneration processes are essential. This further restricts the breadth of the candidate materials and fabrication techniques suitable for applications in TE&RM. Due to such complex design requirements and technical challenges, progress in the development of advanced soft materials for TE&RM has been long-drawn-out.

Soft biological materials are primarily composed of a tough network consisting of high molecular weight fibrous proteins e.g., collagens, fibronectin and elastin, embedded within a hydrated gel-like matrix. These fibers act as the main load bearing element with many biomechanical features of soft tissues attributed to their structural organization and morphology.5 For instance, straightening and progressive tensioning of initially wavy collagen fibers with increasing strain lead to a unique nonlinear stress–strain (σ–ε) response described by a distinct J-shaped curve.6 In addition, fibers are known to be responsible for the mechanical anisotropy of many biological materials.7 Furthermore, the complex biomechanical properties of soft biological materials also arise from the dynamic interplay between the solid and fluid matrix constituents. The underlying mechanisms of strain-rate dependency and stress-relaxation of soft tissues have been associated with the kinetics of the interstitial fluid;8 therefore, composite systems consisting of a water-saturated soft matrix with reinforcing fibers hold great promise to be applied in soft tissue engineering.

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Specifically, hydrogels, water-swollen hydrophilic polymers, can be tuned to mimic the compositional and physicochemical properties of nature’s biopolymer matrices. Concurrently, fibrous constructs with morphology analogous to collagen fibrils can be manufactured to reinforce aforementioned hydrogels leading to mechanically and biologically functional composite systems resembling the structure of soft biological tissues. Owing to these attractive properties and great potential of hydrogel matrix fiber-reinforced systems, several strategies have been reported toward the development of such composites. However, studies reported in the state of the art literature often lack to adequately address all of the

Figure 1. (a) Schematic representation of a MEW device with printing parameters adjusted to obtain a customary jet formation (jet formation #2) needed for accurate printing of complex structures. The influence of the translational collector speed on the shape of the electrified jet is illustrated. (b, c) Field-emission scanning electron microscopy (FE-SEM) images of the 3D printed fibers from the design library with different line spacing (LS), arc radius and curvature degree. (d, e) FE-SEM images of exemplary fibrous networks with (d) biomimetic and (e) control designs. The insets show the superimposed FE-SEM micrographs and the programmed pathway for the assessment of the manufacturing tolerance. (f) Schematic illustration demonstrating how to tune the system parameters to fulfill the design requirements of a tissue-specific soft network composite system. (g) Demonstrations of soft network composites for various soft tissues including skin, anterior longitudinal ligament, masseter muscle and anterior cruciate ligament on 3D printed models with hypothetical fibrous network designs imaged with FE-SEM.
crucial requirements of TE&RM from the design, material, and/or processing point of view (for details see Table S1). For instance, soft network composites consisting of a water-swollen polymer matrix reinforced with lithographically printed networks that closely match the mechanical performance of human skin has been reported. However, this approach is valid for a broad range of medical applications such as stretchable electronics and drug releasing devices, it is not suitable for TE&RM applications because the concept relies on manufacturing methods and materials that do not allow cell embedding and tissue regeneration. Yet, the development of biomaterials for sustained cell viability, functionality and enhanced mechanical behavior is imperative for the design and fabrication of soft network composites as functional soft tissue substitutes. Recently, we have shown that the combination of hydrogels with highly organized printed medical grade poly(e-caprolactone) (mPCL) fibers result in fully biocompatible soft network composites with enhanced compressive mechanical properties making them suitable for articular cartilage tissue engineering applications. However, neither the basic network designs (lay-down patterns of 0°−90° and 0°−60°−120°) based on straight fibers nor the hydrogel matrices used in these composites are proficient of delivering the fundamentally different biomechanical performance required for soft tissues which predominantly function under high tensile loads such as skin, tendon, ligament or heart tissue. In addition, owing to their distinctive fiber architecture and matrix composition, each soft tissue exhibits unique mechanical and biological properties. Hence, the development of a holistic approach which allows the selection and fabrication of tailored tissue-specific reinforcing fiber networks and matrices is required. In this study, we present customizable soft network composites consisting of a hybrid hydrogel system which emulates the native fluid-saturated biopolymer matrices, and 3D printed fibers with organic, stretchable curvy structures intended to mimic the collagen fiber architectures of soft tissues.

First, we create a design library consisting of various 3D printed fibers to be used as the building blocks of reinforcing fibrous networks using an in-house built melt electrosprinning writing (MEW) device (Figure 1a−c). MEW is a fiber processing technique which combines electrosprinning and additive manufacturing principles (see Figure 1a for the schematic illustration of MEW and, S1 and Video S1 for further details).

In the process, a high voltage (HV) is applied to a molten polymer that is extruded through a metallic needle which results in the formation of a polymeric jet. Once this polymeric jet is stabilized by tuning the printing parameters including HV, temperature, pressure, translational collector speed and working distance (Figure 1a), the electrified jet is then printed on a grounded collector in a layer-by-layer fashion using a computer controlled translational stage. As shown in Figure 1b, c, unlike other fiber fabrication techniques such as solution electrosprinning, knitting and weaving, MEW allows the translation of innovative biomimetic design ideas to physically manufactured fibrous networks. The field-emission scanning electron microscopy (FE-SEM) images demonstrate the precise deposition and control on the design features of the fibers including line spacing (LS), arc radius and curvature degree (see Figure S1 for the orientation angle distribution analysis on the fibers). In addition, MEW uses melts of mPCL and circumvents the use of solvents that may compromise the biological properties of the resultant fibrous network.

To demonstrate our approach, we manufactured a conceptual biomimetic fibrous design featuring anisotropy (consisting of curved (flexible) and straight fibers (rigid) in different loading directions) and stretchability (Figure 1d) along with its respective control with straight fibers (Figure 1e) using the elements from the design library. Other conceptual soft network composite designs developed for various soft tissues including skin, anterior longitudinal ligament, masseter muscle, and anterior cruciate ligament are presented in Figure 1f.

Next, although this approach is applicable to a wide range of hydrogel types, we use a tunable interpenetrating polymer network (IPN) system as the hydrogel matrix (Figure 1f) due to the superior mechanical properties (e.g., high flexibility and toughness) of IPNs when compared to their single network counterparts. Yet, the majority of hydrogels, including IPNs, fall short in capturing multiple mechanical functions of biological materials (e.g., anisotropy and depth-dependent mechanical properties, and often strength). It is known that increasing the concentration of the polymer content and intensifying the degree of cross-linking can enhance the mechanical properties of hydrogels. However, it is important to note that such improvements are usually obtained at the expense of biological functionality. For instance, hydrogels with a very dense polymeric network are often associated with restricted cell migration, proliferation, and differentiation because of reduced porosity, diffusivity, and permeability.

Therefore, biological and mechanical functionalities tend to be viewed as “mutually exclusive”. With this in mind, we use a relatively soft hydrogel (tensile modulus of IPN: \( E_{IPN} = 25.08 \pm 4 \) kPa) and embed high-modulus fibers of mPCL (measured tensile modulus of mPCL fibers: \( E_{mPCL} = 309.50 \pm 46 \) MPa) for reinforcement. mPCL is a biodegradable polyester that is known to exhibit a slow degradation rate and ability to retain its mechanical properties for up to 12 months. We use poly(ethylene glycol) diacrylate (PEGDA) (15% (w/v)) as the primary constituent of the IPN, and gelatin methacryloyl (GelMA) (1% (w/v)) and alginate (1% (w/v)) for enhanced biological and mechanical functionality, respectively. The selected composition allows for good cross-linking leading to a soft yet mechanically robust hydrogel. A strong emphasis is placed on maintaining the polymer content of the hydrogel low to enhance the in vitro and in vivo performance. Specifically, this hydrogel is designed to leverage the elasticity of covalently cross-linked terminally functionalized high molecular weight PEGDA (20 kDa) with the energy dissipation capability of reversibly cross-linked alginate.

We incorporate chemically functionalized gelatin (GelMA) to introduce natural cell-binding and provide protease-cleavage sites that are missing in the majority of reported IPN systems. The resultant hydrogel system is entirely composed of tunable, biodegradable and biocompatible materials that address all the desired design requirements (Figure 1f), as well as allow for cell encapsulation. It is known that the interface between the matrix and reinforcing fibers of a composite is critical to effectively transfer the applied loads from the soft matrix to high-modulus fibers. Moreover, fibers with high aspect ratios increase the interfacial surface area between the composite constituents. Therefore, we print reinforcing networks with an average fiber diameter of 23 ± 0.9 μm with high reproducibility throughout both designs and then modify their surface by grafting photo-cross-linkable acryl groups (see S11 for further information). This functionalization provides a site for covalent bonding between
the hydrogel (PEGDA and GelMA) and fiber surface (Figure 2a). We validate the functionalization of the mPCL fiber surface by cross-linking acrylated fluorescein. As shown in Figure 2b, acrylated fluorescein exhibits enhanced binding to the modified mPCL surface (see SI1 and Figure S2 for further information). Surface modification of the fibers is also confirmed via X-ray photoelectron spectroscopy analysis (see SI1 and Figure S3 for further information). Moreover, the adhesion strength of the hydrogel matrix to methacrylated and untreated mPCL fibers is evaluated using a microbond test where a droplet of hydrogel (PEGDA and GelMA) is placed in a CL-1000L ultraviolet (UV) cross-linker. Photo-cross-linking of PEGDA and GelMA hydrogels is achieved by exposure of UV (wavelength of 365 nm) for 10 min, and alginate is ionically cross-linked with calcium sulfate (CaSO4) (see SI1 for further information). Stereomicroscope images of the resultant soft network composites are shown in Figure 3a.

We perform uniaxial tensile tests at a displacement rate of 0.1 mm/s to characterize the mechanical properties of our composites (after 24−36 h of incubation in Dulbecco’s Modified Eagle Medium (DMEM) at 37 C° (n = 6) (see SI1)). Initially, individual composite constituents (hydrogel and bundle of single mPCL fibers) are tested. Mean σ−ε curves of hydrogel (tensile modulus of IPN EIPN = 25.08 ± 4 kPa) and mPCL fibers (tensile modulus of mPCL fibers E200 = 309.50 ± 46 MPa) are shown in Figure 3b and c. Although it is not very prominent, the tests reveal that the hydrogel exhibits nonlinearity (Figure 3b and Video S3), whereas mPCL fibers display a σ−ε curve similar to that of a typical flexible plastic where a large elongation at near constant stress is observed after the material yields (Figure 3c). Next, we test our soft network composites with both biomimetic and straight fibrous network designs (Video S4). Experiments reveal that the σ−ε profile of the composite with straight fibers is comparable to that of mPCL fibers. As the straight fibers of the control composite are already taut, a rapid increase is observed on the stress axis of the σ−ε curves with applied tensile strains (Figure 3d). In contrast, composites with bioinspired fibers display a J-shaped σ−ε curve (up to a strain of ~0.40) with three distinct phases (toe, heel and linear) resembling those of biological soft materials (Figure 3e). Similar to the collagen fibrils of biological materials, a gradual fiber realignment and tensioning is observed (Video S4).

In an effort to gain greater mechanistic insight on the deformation mechanics of our composites, we develop a numerical model employing the p-version of the finite element model (p-FEM) to complement the mechanical tests. This strategy differs from the approach that is widely implemented in commercial FEM packages where the number of the mesh elements is increased to improve accuracy. Instead, in p-FEM, the polynomial degree of the elements is increased for closer approximation and therefore confers several advantages when simulating elements with high aspect ratio, such as fibers (e.g., less computational cost and reduced solver time).31 Despite these benefits, the potential of p-FEM is yet to be recognized in the field of TE&RM. In our analyses, we use a polynomial degree of three (see SI1 and Figure S5). We use two-term compressible Mooney-Rivlin hyperelastic material model32 to describe the hydrogel matrix and the deformation theory of plasticity33 for the reinforcing fibers. Figure 3b, c compares the mean of experimentally determined σ−ε curves of the hydrogel and mPCL fibers to those obtained with the simulations. The Mooney−Rivlin hyperelastic model is able to predict the nonlinear deformation behavior of the hydrogel in the uniaxial testing arrangement with sufficient accuracy (results lay inside the error bars) at large strains (up to ε ∼ 0.65) (see Video S5). With regards to mPCL fibers, our p-FEM model manages to capture the yield point, linear elastic and plastic regions of the σ−ε curves of straight mPCL fibers in good accordance with the experimental results (up to a ε ∼ 0.125) (see Video S6).
Next, we perform the simulations on our soft network composites (Figures 3d and 4b, c). Even though the results do not lie within the error bounds of experimental findings, we are capable of capturing the shape of the $\sigma-\varepsilon$ curves which closely resembles the test data. It is likely that idealization of the computational model as well as variabilities in experimental conditions in the physical testing setting contribute to the variances in the $\sigma-\varepsilon$ curves. As anticipated, soft network composites with straight fibers exhibit a near identical deformation characteristic to that of straight fiber bundles. Our computational findings suggest that the fibers in this design undergo plastic deformation after an axial strain of $\sim 0.05$ (see Video S7). However, no plastic deformation is detected on the fibers of the composites with biomimetic design up to a strain of $\sim 0.45$ (see Video S8). In this case, fibers uncoil to a strain of $\sim 0.45$ before experiencing intense stresses. This uncoiling ratio is comparable to the physiological stretching capacity of many soft biological materials. The majority of soft tissues can withstand maximum tensile strains of 0.30–0.40 before irrecoverable damage ensues (e.g., $\varepsilon$ at ultimate tensile stress of skin is $\sim 0.30$; $\varepsilon$ at failure of ligament, tendon and heart valve leaflet are $\sim 0.15$, $\sim 0.30$, and $\sim 0.30$, respectively). In our biomimetic soft network composites, irreversible deformation occurs only on the fibers after they are fully taut ($\sim 0.45$) and stretched beyond the yield point. The design of the fibrous networks can be tailored to match the stiffness and stretching capacity of the intended tissue (see Figure S6 for $\sigma-\varepsilon$ curves of soft network designs with different pore sizes, fiber diameters and degree of curvatures obtained via simulations).

Figure 3. (a) Custom-made molding system used for the preparation of the soft network composites with the stereomicroscope images of the resulting constructs after incubation in cell media (DMEM). (b–d) Averaged stress–strain curves of (b) hydrogel, (c) mPCL fibers and (d) soft network composites obtained from the experiments in comparison to those from the simulations. (e) Averaged stress–strain curve of hydrogels in comparison to those of hydrogels reinforced with bioinspired fiber design. The area under the curves is used to calculate the work of extension of the tested specimens.
In the physical experiments, because of the high molecular weight of mPCL (95−140 kDa, PURASORB PC12, Corbion Purac, The Netherlands), despite extensive stretching, failure does not occur with increasing strain within the working distance of the mechanical testing system (axial tensile ε > 2.5) (W\text{Ext Composite} = 2026 ± 250 kJ m\textsuperscript{−3}). However, partial disintegration is observed on the hydrogel matrix most likely due to shear related deformation at a strain of ∼0.5. IPN hydrogels fail at an axial tensile strain of 1.28 ± 0.13 (W\text{Ext Hydrogel} = 20.65 ± 3.0 kJ m\textsuperscript{−3}). We measure the tensile modulus (E) of the bioinspired soft network composite to be ∼125-fold higher than that of hydrogel samples (3.19 ± 0.3 MPa vs 25.08 ± 3.8 kPa).

Preliminary in vitro experiments suggest that our soft network composites are cell biocompatible and support the viability of encapsulated human bone-marrow derived mesenchymal precursor cells (hBMPCs). We measure the viability of hBMPCs in soft network composites with biomimetic and control fiber designs after 72 h to be 80 ± 3% and 86 ± 12%, respectively, with no statistical difference (p < 0.05) (Figure S7a, b). Therefore, hydrogels with varying biomaterial composition and biomechanical properties designed for specific TE&RM applications can be readily developed. In addition, biological functionality of these tissue engineered constructs (TECs) can be enriched by incorporating various biomolecules, growth factors and other extracellular matrix proteins within the hydrogel component of the system.

In summary, we develop and characterize an integrated design, material, and fabrication platform for the development of soft network composites for engineering tissues functioning under high tensile loads. The platform allows for tuning of the most relevant biomechanical and biological properties of fabricated soft network composites. By combining different elements from the design library, composites with regionally and globally controlled complex deformation behaviors can be developed. More importantly, the proposed in silico model is developed as a design tool to reduce the time and effort dedicated to physical fabrication and characterization of future soft network composites, which inherently have an infinite number of different designs. In conclusion, on the basis of these results, we foresee the translation of the concept into a wide range of soft tissue engineering applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.7b08617.

Experimental details (PDF)

Video S1, melt electrospinning writing process (AVI)
Video S2, microdrop test (AVI)
Video S3, uniaxial testing of hydrogel (AVI)
Video S4, uniaxial testing of soft network composites (AVI)
Video S5, simulation of hydrogel (AVI)
Video S6, simulation of mPCL fiber (AVI)
Video S7, simulation of soft network composite (control with straight fibers) (AVI)
Video S8, simulation of soft network composite (biomimetic design) (AVI)

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O.B., E.M.J.P., and D.W.H. designed the experiments and conceptualized the manuscript. O.B. conducted the experimental work and wrote the manuscript. D.D., S.K., A.R. and H.M. developed the numerical model. J.G.B. performed the in vitro work. N.J.C., F.M.W., and N.T.S. assisted the experimental work. All authors provided feedback and D.W.H. assigned final approval to the manuscript.

Notes

The authors declare no competing financial interest.

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