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Elastomeric Cell-Laden Nanocomposite Microfibers for Engineering Complex Tissues

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Abstract—Biomaterials-based three dimensional scaffolds with tunable elasticity hold promise in replacing failed organs resulting from injuries, aging, and diseases by providing a suitable cellular microenvironment to facilitate regeneration of damaged tissues. However, controlled presentation of biological signals with tunable tissue mechanics and architecture remain a bottleneck that needs to be addressed to engineer functional artificial tissues. Nanocomposite hydrogels that promote cells adhesion and demonstrate tunable viscoelastic properties could mimic key properties and structures of native tissue. We have developed elastomeric fiber shaped cellular constructs from poly(ethylene glycol) diacrylate, silicate nanoparticles, and gelatin methacrylate *via* ionic and covalent crosslinking. By controlling the interactions between nanoparticles and polymers, nanocomposite hydrogels with tunable mechanical and degradation properties are fabricated. By encapsulating multiple cell types in these cellular constructs, we demonstrate materials-based control of cell spreading, survival, and proliferation. As a proof-of-concept, we assembled the hydrogel microfibers to obtain multicellular elastomeric tissue constructs. These elastic microfibers may serve as model systems to explore the effect of mechanical stress on cell–matrix interactions. Moreover, such elastomeric hydrogel fibers can be used to engineer scaffold structures, fabric sheets, bundles, or as building blocks for 3D tissue construction.

Keywords—Nanocomposite hydrogels, Nanoparticles, Microfibers, Cell–matrix interactions, Tissue engineering, Bioadhesive.

INTRODUCTION

Complex tissue structures are difficult to mimic from a biomedical engineering perspective due to the complexity associated with its native structure at multiple length scales. Often, biomaterials are developed as stand-alone implants with bulk properties and interdependent biophysical properties such as bio-adhesivity and/or mechanical strength. As biomaterial

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design progresses, biomimetic design can address some of the challenges needed to engineer complex tissue.[12,17,18,37] The need to mimic the tissue architecture along with the ability to control or enhance cell adhesion and proliferation is a challenge to traditional tissue engineering approaches. Many complex tissues such as bone, muscle, tendon and ligament have microarchitectures that have not yet been efficiently recapitulated using existing biomaterials.

Hydrogel chemistry and fabrication has quickly morphed into a paradigm for tissue engineering since its inception.[23,34] Mechanically robust synthetic systems are often sought and studied, while naturally occurring polymers are inadequate to fully meet the mechanical requirement for implantation. Poly(ethylene glycol) (PEG) diacrylate has demonstrated biocompatibility in a wide variety of biomedical applications spanning drug delivery to tissue engineering.[2,32–35] However, due to its inert chemical composition, PEG in its native composition has limited applications as a scaffolding material.[16,25,28] In order to fully take advantage of the PEG network, supplementary additives are used. Natural polymers, like gelatin (Gel), enable cell-driven degradation to augment migration throughout the matrix and also provide unique amino-acid sequences for mediated binding to the substrate.[19,29,39] However, Gel cannot covalently crosslink in networks; therefore, gelatin methacrylate (GelMA) is used to obtain covalently crosslinked Gel.[19] Gel provides advantages in processing when compared to collagen semi-interpenetrating hydrogels or polymer backbones that use degradable or biologically relevant sequences.[20,31,41] In this study, we have focused on incorporating Gel within PEG hydrogels to modulate cell adhesion and improve the biomaterial degradation characteristics. This is an advantage over current methods due to the simplicity of design.

Recently, there has been significant interest in manufacturing bioadhesive hydrogel matrices[3,22]; including nanocomposite hydrogels[8,15] that offer unique advantages over conventional single polymer hydrogels. For example, to design bioactive hydrogels for bone regeneration, bioactive nanoparticles such as hydroxyapatite, synthetic silicates or bioactive glass can be incorporated with in polymeric matrix.[2,21] Recently, we have reported that synthetic silicates, a class of ultrathin nanomaterials, incorporated within Gel hydrogel can upregulate production of osteo-related proteins and deposition of mineralized extracellular matrix.[11,26,42] In addition, we have also reported that Silicates when incorporated into a poly(ethylene oxide) (PEO) matrix can control cell adhesion, spreading and proliferation.[13,14] Due to their unique shape and surface charge characteristic, Silicate nanoparticles interact with Gel[9,42] and PEG[10] resulting in shear-thinning and viscoelastic network. Silicate loaded hydrogels are biocompatible and biodegradable under *in vivo* conditions and can be used for a range of biomedical and biotechnological applications.[9] Recently, using Michael-addition based reactions and Silicate nanoparticles with Gel an interpenetrating network (IPN) that enabled independent control over adhesive ligand availability and tissue stiffness for tumor cell engineering was created.[30,36]

Here, we engineer multicomponent semi-IPN hydrogels as elastomeric microfibers with enhanced cell adhesion characteristics. By combining PEG with Silicates nanoparticles, elastomeric and mechanically stiff microfibers can be engineered that allow mimicking viscoelastic characteristic of native tissues. The addition of Gel to PEG/Silicates provides additional control over degradation properties. By designing this multi-component system, we propose to engineer cell adhesive biomaterials with elastomeric properties. Even more so, we aim at designing a simple system that allows for easy mechanical modulation. It is our hypothesis that by using the charge differences between the Silicates and aqueous solutions, that complex structures can be generated. By exploiting this characteristic, we hypothesize the use of a simple PEG/Gel/Silicate solution to encapsulate cells for biomedical applications. Applications for such a system, especially a fiber hydrogel system, ranges from nerve tissue regeneration to blood vessel formation; additional complex geometries could be obtained through use of three dimensional printer capabilities.

MATERIALS AND METHODS

Materials

Synthetic silicate nanoparticles (Silicates) (Laponite XLG) were obtained from BYK-Chemie GmbH (Wesel, Germany). PEG (M_W = 10 kDa), Gel (300 g Bloom, type A) from porcine skin, and methacrylic anhydride were purchased from Sigma-Aldrich (St. Louis, MO). Acryloyl chloride and triethylamine were purchased from Alfa Aesar (Ward Hill, MA). Poly(ethylene glycol)-diacrylate (PEG) and gelatin methacrylate (Gel) were synthesized as previously described.[4,29] For proliferation studies, preosteoblast cell type MC3T3-E1 Subclone 4 were used (ATCC®CRL-2593). Fluorescent cell types for encapsulation visualization were a transfected green fluorescent protein (GFP) NIH 3T3 preosteoblast from Cell Biolabs (San Diego, CA) and a red fluorescent protein (RFP) transfected mouse osteosarcoma (mosJ) obtained from Dr. Roland Kaunas' Lab, Biomedical Engineering Department, Texas A&M University.

Nanocomposite Formulation

Stock solution of 8 % (w/v) Silicate was prepared in milliQ water by vigorous agitation at 4 °C. Ciba® IRGACURE® 2959 (Ciba Specialty Chemical, USA), UV photoinitiator (PI), stock solution of 0.6 % (w/v) was prepared in milliQ water. The nanocomposite pre-polymer solution were fabricated by vortexing Silicate stock with PI stock and dissolving in 5 % PEG (10 kDa) and 1 % Gel. Vortexing and centrifugation ensure proper mixing of solution. The nanocomposite pre-polymer solution was injected in 10× phosphate buffer saline (PBS) using glass micropipette (Drummond) of different diameters to obtain physically crosslinked microfibers. These microfibers were subjected to ultra-violet (UV) radiation (320–500 nm) (Omnicure S200, Lumen Dynamics, Canada) for 10 s at an intensity of 8 mW/cm^2. PEG and PEG/Gel solutions do not form fiber structures when placed in PBS or media.

Chemical Characterization

Attenuated total infrared reflection (ATIR) was performed using Bruker vector-22 FTIR spectrophotometer (PIKE technologies, USA) on all dried hydrogel compositions. Zeta potentials and hydrodynamic size of PEG, PEG/Gel, PEG/Silicates, and PEG/Silicates/Gel precursor solutions were determined in ultrapure water (Milli-Q) using a 633 nm laser in a Malvern ZEN3600 (Malvern Instruments, UK). Scanning electron microscopy (SEM) images of the lyophilized hydrogels were obtained using FEI Quanta 600 FE-SEM, USA fitted with Oxford EDS system at an accelerating voltage of 20 kV.

Nanocompostie Hydration and Degradation

Nanocomposite samples were placed in 1.5 mL Eppendorf tubes and weighed. Each sample was frozen, lyophilized and weighed. Comparing the wet weight to dry weight, hydration degree was calculated. Accelerated degradation was performed in presence of collagenase Type II (Worthington Biochemicals, USA) solution (5 U/mL) in PBS at 25 °C while shaken. Comparison of fully hydrated wet weight to collagenase treated wet weight determined the mass% remaining.

Mechanical and Rheological Analysis

An ADMET eXpert 7600 Single Column Testing System equipped with 25 lb load cell was used for compression testing. Strain rate of 1 mm/min was used to compress the samples 50 % of original height. The compressive modulus was calculated and plotted vs. the sample composition. An Anton Paar MCR 301 rheometer was used for mechanical testing. Parallel-plate geometry (10 mm diameter) with a gap height of 400 μm was used for UV rheology. The UV light was turned on after 30 s with an intensity of 8.4 mW/cm^2. Stress was set constant at 1 Pa and strain constant at 1 Hz. Viscosity of precursor solutions were measured at a gap height of 400 μm.

In vitro Studies

For cell spreading, preosteoblasts (NIH MC3T3 E1-4, ATCC, USA) were seeded onto UV sterilized bulk polymer compositions in a 96-well plate at a density of 10,000 cells/mL. At each time point, cells were washed in PBS, fixed with 2 % glutaraldehyde for 12 min at room temperature, permeated with 0.1 % Triton X-100 for 5 min, then incubated with rhodamine phalloidin for 20 min at 37 °C followed by DAPI for 5 min. The gels were removed from the wells and inverted onto glass coverslips for fluorescence imaging. Cell area was calculated using ImageJ (NIH, Bethesda, MD) and normalized with number of cell nuclei. For encapsulation, prepolymer solutions were physically mixed with a concentrated cell pellet of interest. The solution was then collected in a 1 mL syringe and extruded into media, in which the fibers would maintain shape due to physical crosslinking. UV light was then utilized for further crosslinking as previously explained. An additional cell type could be added to the surface of the fibers *via* adding a cell suspension dropwise to a petri dish containing media and fibers with encapsulated cells. Encapsulated preosteoblast viability was evaluated using Calcein AM and Ethidium Homodimer staining (Life Technologies, Carlsbad, CA). Cell cycle analysis was performed using the BD Accuri C6 Flow Cytometer and propidium iodine (PI) stain following manufacture's protocol. In short, cells seeded on each composition were trypsinized at two separate time points and fixed in cold 70 % ethanol. Cell pellets were formed and washed in PBS, followed by incubation in a PI staining solution at 37 °C for 30 min. Cells were stored at 4 °C until analysis. Focal adhesion studies were performed be encapsulating mouse embryonic fibroblasts expressing vinculin fused to eGFP (vinculin T12) in PEG hydrogels.[6,7] Vinculin localization was imaged using fluorescence microscopy. Co-culture of cells were performed by encapsulating RFP-mosJ cells within the microfibers and seeding GFP-3T3 cells on the microfiber surface.

Statistical Analysis

Determination of statistical significance between multiple groups was completed *via* analysis of variance (ANOVA) with Tukey method. Significant p values

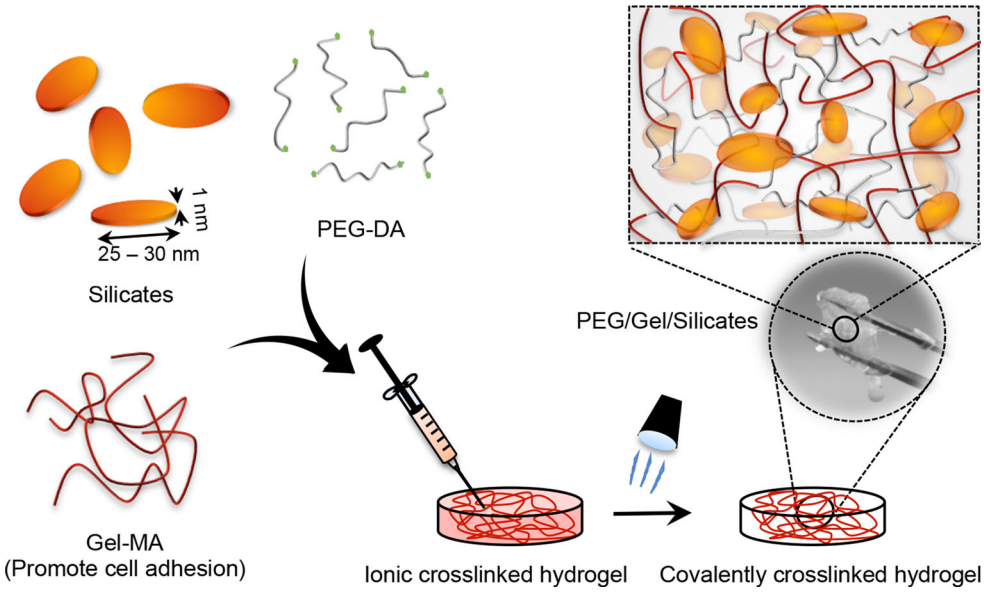

FIGURE 1. Synthesis of nanocomposite microfiber hydrogels *via* ionic and covalently crosslinking. Silicate nanoparticles are combined with PEG and Gel and injected into PBS to obtain ionically crosslinked microfibers. UV crosslinking for 10 s results in formation of covalently crosslinked network. Covalently crosslinked microfibers are mechanically stiff and elastomeric.

were considered <0.05 unless otherwise noted. All analysis was completed in GraphPad Prism (San Diego, CA).

RESULTS AND DISCUSSIONS

The nanocomposite hydrogels were fabricated by mixing prepolymer solution containing PEG, Gel, and Silicates (Fig. 1). Using ionic interactions, covalent crosslinking chemistries, and viscosity, we have been able to extrude fibers of various sizes ranging from 0.2 to 1 mm. By using various strengths of PBS (1–20×) we found that 10× PBS forms the most ionically stable hydrogels (physical crosslinked). Therefore, for all sample preparation, 10× PBS was used. Hydrogels were mechanically stable after UV curing (chemical crosslink) and could be assembled into knots and other complex geometries simply using tweezers.

Physical Characterization of Nanocomposite Solutions

The prepolymer solution of PEG and PEG/Gel remained liquid at room temperature (25 °C) (Fig. 2a). Addition of Silicates to these prepolymer solutions resulted in formation of highly viscous solutions. The interactions between Silicate and polymers were investigated using electrophoretic measurements and DLS. The zeta potential of prepolymer solution of PEG and PEG/Gel was -5 ± 0.4 and -7.1 ± 0.6 mV, respectively and Silicates was -28.7 ± 2.4 mV. The addition of Silicates to prepolymer solutions resulted in a decrease in zeta potential of PEG and PEG/Gel to -24.2 ± 1.2 and -27 ± 1.9 mV, respectively (Fig. 2b). We next determined the effect of Silicate size with polymer addition. The hydrodynamic diameter of Silicates was ~56 nm, and the addition of PEG and PEG/Gel prepolymer to Silicates increased the hydrodynamic size of silicates to ~65 and ~72 nm respectively (Fig. 2c). These findings suggest that polymer coats the Silicate nanoparticles before crosslinking. Silicates naturally exfoliate when placed in prepolymer solution and result in an increase in viscosity of precursor solutions. With an increase of shear rate, we observed a decrease in viscosity for all compositions (Fig. 2d); addition of Silicates increased the viscosity when compared to non-Silicate containing compositions. The shear-thinning characteristic was used to fabricate microfibers with specified diameter. The ionic interactions between Silicates present on the microfibers and ions present in PBS, result in formation of ionic complex, stabilizing the surface of microfibers. After exposing the prepolymer solution to UV, covalently crosslinked network was formed, indicated by the increase in G' (Fig. 2e).

PEG is a hydrophilic polymer and covalently crosslinked PEG hydrogels contains around 97 % water. The hydration degree of PEG hydrogels decreases with the addition of Gel and Silicates (Fig. 3a). This might be attributed to higher crosslinking density due to addition of Gel and Silicates. Despite high degree of water content, nanocomposite hydrogel net-

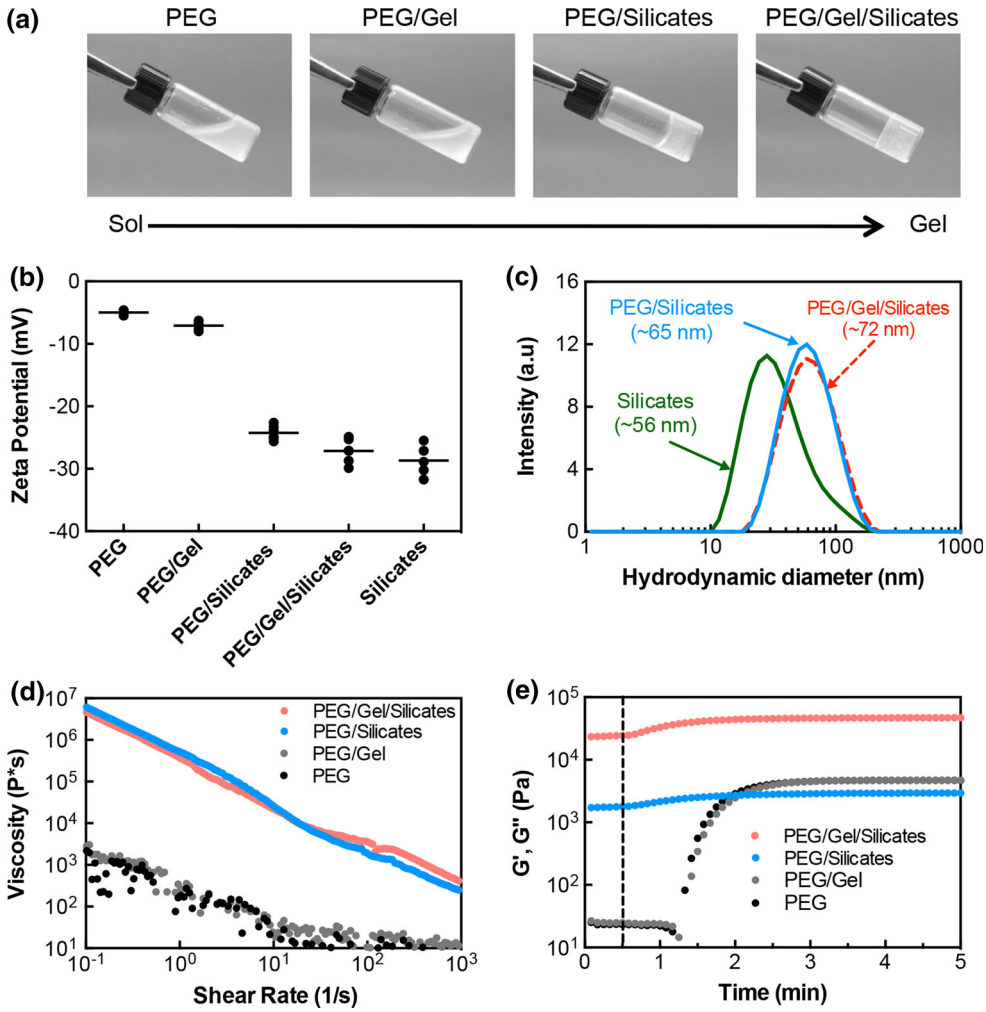

FIGURE 2. Shear thinning characteristic of prepolymer solution. (a) Viscosity of prepolymer solution increases due to addition of Silicate nanoparticles. (b) Zeta potential and (c) dynamic light scattering of prepolymer solutions indicate that Silicates strongly interacts with PEG and PEG/Gel. (d) Shear-thinning characteristic of prepolymer solutions was investigated by monitoring viscosity with respect to shear rate. (e) UV rheology of precursor solutions indicates that addition of Silicates result in stronger network.

work is highly elastomeric as demonstrated by formation of hydrogel knot (Fig. 3b). The addition of Silicates inhibited the uptake of water into the PEG/Gel/Silicates hydrogel and was significantly different ($p < 0.001$) from PEG and PEG/Gel hydrogels. This can potentially be attributed to the Silicates being fully exfoliated before PEG and Gel were added.

The addition of Silicates or Gel to PEG had no effect of the mechanical stiffness (Fig. 3c). However, the compressive modulus was significantly increased for PEG/Gel/Silicates to 6.5 ± 1 kPa compared to PEG (1.7 ± 0.1 kPa), indicating synergistic effect of Silicates and Gel. The addition of Silicate to PEG/Gel composition appears to modulate the mechanical properties of nanocomposite hydrogels. A linear correlation was observed between the concentrations of silicate nanoparticles and modulus of the nanocomposite hydrogels. However, addition of lower amount of silicate (<4 %) does not result in formation of stable microfibers. Thus, we selected nanocomposite loaded with 4 % Silicates for further characterization. All the hydrogel samples sustain severe compression loading and unloading cycle indicating elastomeric behavior (Fig. 3d). The addition of Silicates and Gel, resulted in significant increase in ultimate stress at 50 % compression.

PEG is a non-degradable hydrogel and the addition of Gel to PEG results into formation of composite network that can undergo enzymatic degradation. The degradation of hydrogel networks is highly desirable to facilitate cell spreading, cellular infiltration and tissue growth. We observed that when collagenase solution was added to fully hydrated samples, there was a decrease in wet weight for samples containing Gel

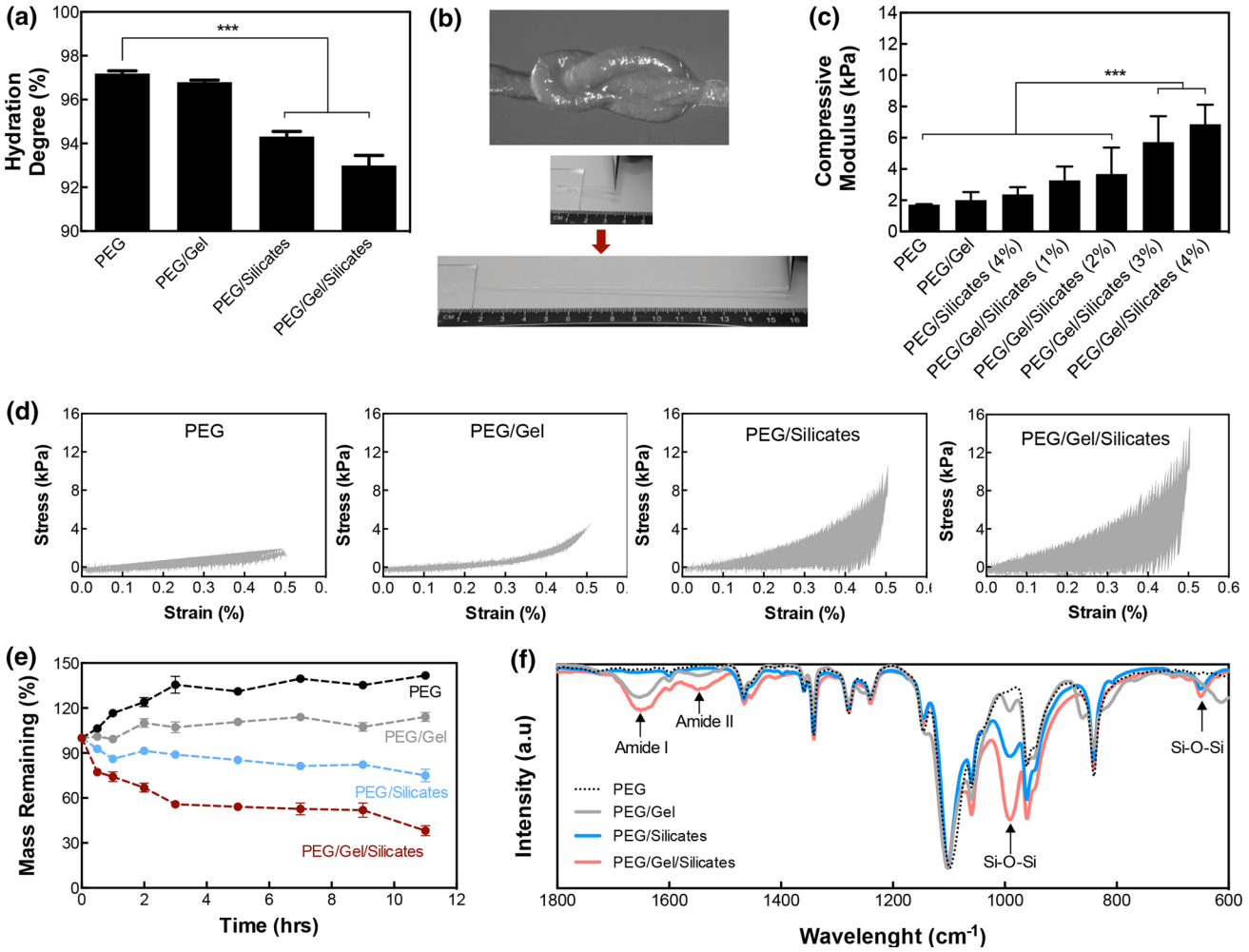

FIGURE 3. Physical stability of nanocomposite hydrogels. (a) Hydration degree (%) of PEG, PEG/Gel, PEG/Silicates, and PEG/Gel/Silicate hydrogel samples are shown. The addition of Silicates result in a significant decrease in the saturated hydration degree due to the strong interactions between Silicate and polymer chains (*$p < 0.005$). (b) Highly elastomeric hydrogel fibers can be fabricated from PEG/Gel/Silicate (4 %). (c) Compressive moduli of hydrogel samples indicate addition of Silicate to PEG/Gel hydrogel result in threefold increase in modulus. Silicate concentration can modulate the mechanics. (d) Representative cyclic compression curves for all compositions are also shown. (e) Degradation study of PEG/Gel and PEG/Silicates/Gel. (f) FTIR analysis of lyophilized nanocomposite samples indicate presence of Amide I (1650 cm^{-1}), Amide II (1530 cm^{-1}) and Si–O–Si peaks (1030 and 650 cm^{-1}), indicating presence of gelatin and silicates.**

(Fig. 3e). We observed no significant difference in weight for samples not containing Gel (PEG and PEG/Silicates). A significant degradation was observed in PEG/Gel/Silicate hydrogels. Within 4 h, the hydrogels network dissociated to smaller fragments and within 12 h, PEG/Gel/Silicate hydrogels was completely dissociated. We believe that the low percentage of Gel is biodegraded through collagenase solution and left over is PEG and Silicates. Gel has multiple covalent binding sites for PEG and as the Gel was biodegraded, we observed the network physically disassembling. For implantation, this property is important as to facilitate natural tissue ingrowth.

The presence of Silicate and Gel within the PEG network was confirmed *via* FTIR spectra and EDX analysis. The FTIR spectra indicate the presence of Gel and Silicate in nanocomposite hydrogels (Fig. 3f). FTIR analysis of lyophilized nanocomposite samples indicate presence of Amide I (1650 cm^{-1}), Amide II (1530 cm^{-1}) and Si–O–Si peaks (1030 and 650 cm^{-1}), indicating presence of Gel and Silicates.

The microstructure of PEG, PEG/Gel and PEG/Gel/Silicates indicated that addition of Gel or Silicates enforced formation of porous and interconnected network (Fig. 4a). The nanocomposite fibers (PEG/Gel/Silicates) also showed formation of highly porous and interconnected network (Fig. 4). The average pore size of nanocomposites was ~17.6 μm. The uniform distribution of Silicates within nanocomposite hydrogels was determined using EDX mapping of silicon and

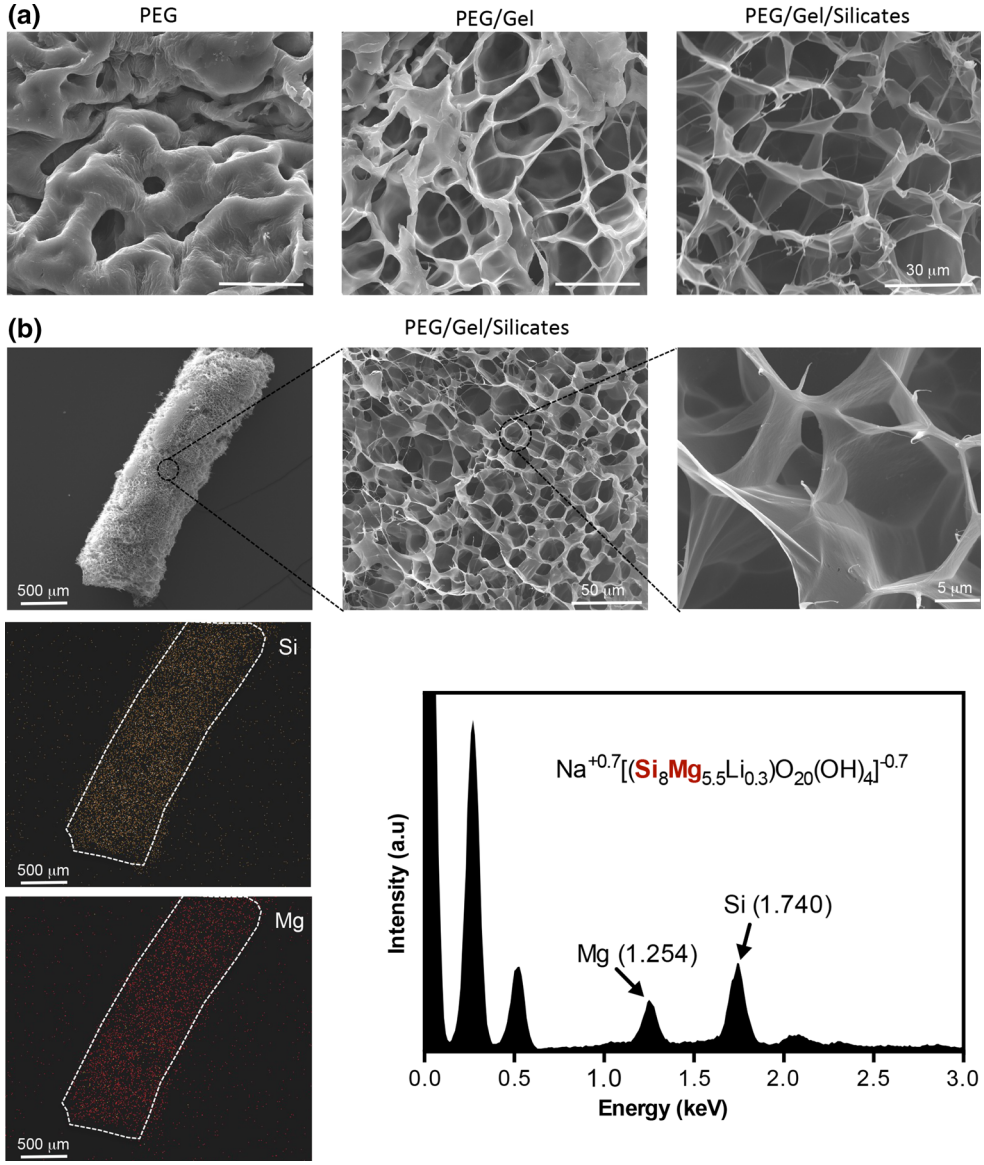

FIGURE 4. Microstructure characteristics of nanocomposite fibers. (a) Representative SEM images for PEG, PEG/Gel and PEG/Gel/Silicates. (b) PEG/Gel/Silicate microfiber show highly porous and interconnected network at different magnification. EDX mapping of silicon (Si) and magnesium (Mg) within PEG/Gel/Silicate fiber indicate uniform distribution of Silicate nanoparticles within the hydrogel structure.

magnesium. No aggregation of Silicates was observed within the hydrogel network indicating strong interaction between polymer and nanoparticles.

Cell-Nanocomposite Interactions

Cellular adhesion to biomaterial scaffolds plays a vital role in the survivability, proliferative capacity and development of new tissues. To examine the biological impact of incorporated Silicates as well as natural polymer, we introduced a variety of model cell types into two-dimensional (2D) and three-dimensional (3D) microenvironments. We hypothesized that the inclusion of Gel and/or Silicates enables enhanced survival and proliferation *via* an interactive cellular environment to improve upon the inert matrix provided by PEG. To investigate cell-Silicates interactions, we evaluated cellular adhesion and spreading on bulk polymeric hydrogels (Fig. 5a). Cells did not adhere to PEG surfaces, as no cell binding sites were present. Interestingly, as we introduced the Silicates to PEG cellular adhesion and spreading significantly improved and was comparable to PEG/Gel/Silicate. These findings suggest that Silicates enables cell spreading which could possibly be attributed to the presence of magnesium in Silicates that supports integrin clustering

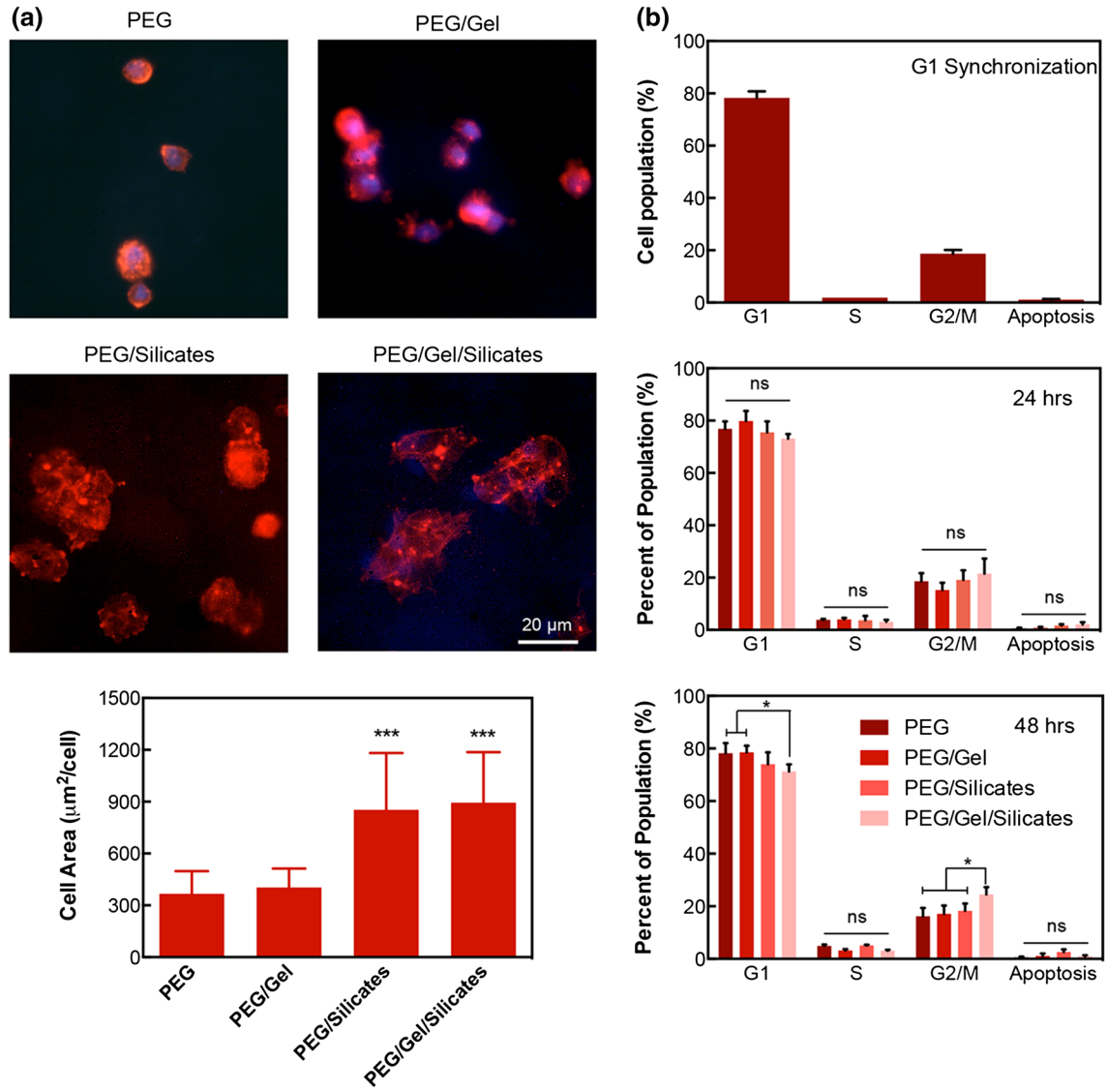

FIGURE 5. Cellular adhesion, spreading and proliferation on nanocomposite hydrogels. (a) Cell adhesion and spreading on hydrogel compositions. (b) Cell cycle analysis of adherent cells at 24 and 48 h indicate addition of Silicates promote cellular proliferation.

and the ability of Silicate nanoparticles to sequester serum proteins.

To further investigate proliferative capacities of the various hydrogel compositions, cell cycle analysis *via* flow cytometry was completed. Initially, cells were synchronized *via* serum reduction for a 24 h period, enabling presentation of a more robust material effect after cell seeding. Incubation on the materials for 24 and 48 h provided ample time for trends to emerge among cell populations. Since PEG is inert, it provided negligible proliferative support and cells remained in the stationary phase (G_0) as demonstrated by the cell cycle analysis (Fig. 5b). 2D seeding of hMSCs onto the various compositions demonstrated that after 24 h, cells seeded on the PEG/Gel/Silicates composition began to display enhanced proliferation, and after 48 h, a significant increase in the G_2/M population was observed, signifying a shift into a higher proliferative capacity. As expected, this was accompanied by a decrease in the G_1/G_0 population as cells entered a mitotic state. RGD sequences within the natural polymer promote cellular adhesion and subsequent cellular spreading, likely leading to the augmentation of cell numbers. Electrostatic interactions between the surface of the nanoparticle and proteins may also provide anchor sites for cell binding events, an additional mechanism for cellular attachment and augmentation of cell survival. Likewise, migration throughout could be mediated by these sites in addition to those enzymatically cleavable locations within the Gel backbone.

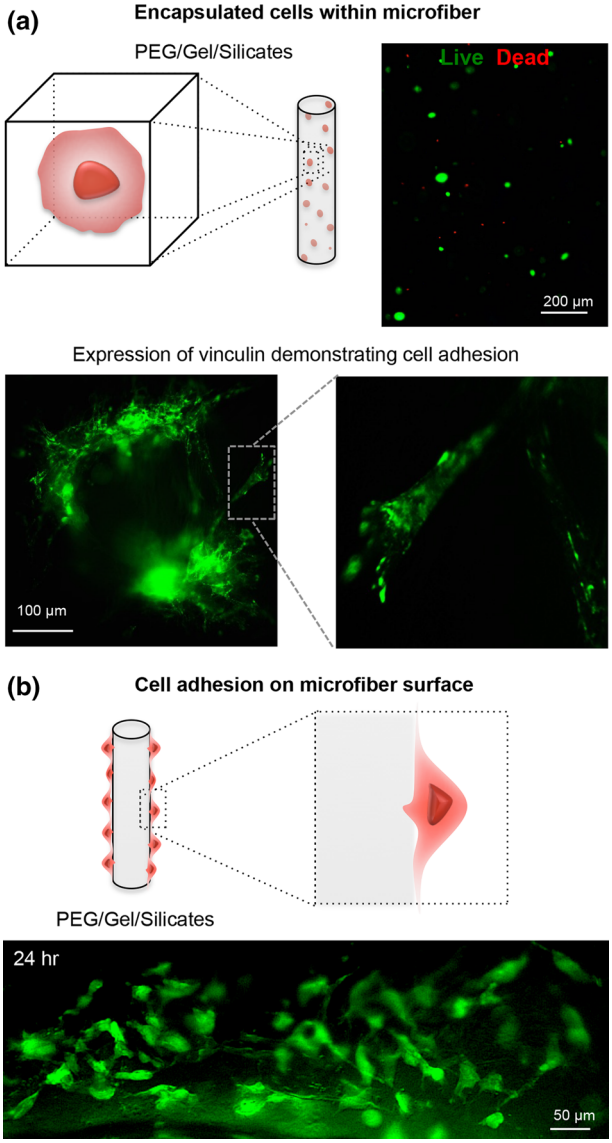

FIGURE 6. Cell seeding and cell encapsulation on hydrogel microfibers. (a) Live/Dead assay showing high cell viability of encapsulated cells in PEG/Gel/Silicates microfibers. Expression of vinculin of encapsulated cells demonstrating cell adhesion. (b) Cells seeded on fiber surface show cell spreading due to presence of Gel and Silicates.

This aspect can be interesting to investigate the formation of tissue structure by seeding multiple cell types.

Hydrogel Microfibers for Engineering Multicellular Structures

With the development of complex tissue scaffolds, nutrient exchange was of utmost importance.[1] It is possible to control the diameter of the fibers by changing the microcapillary diameter during the fabrication process. Cells were encapsulated in precursor solutions and then extruded in either PBS or media. It is expected that the small fiber diameter (<0.5 mm) facilitated fast and efficient nutrient transport.

Cells were encapsulated within the microfibers and viability was assessed to evaluate the effect of fiber formation process on cells. The result indicates that most of the cells encapsulated within fiber survive (viability ~86 $\pm$ 5 %) the fabrication process and very few apoptotic cells were observed (Fig. 6a). Due to the small diameter of the fibers ($\leq$500 μm), diffusion of vital nutrients was not hindered, and therefore cells within the core of the fiber were alive. Furthermore concerns regarding shear stresses placed on cells during extrusion and UV exposure for crosslinking showed no adverse effect on cell viability. Cellular adhesion in 3D microenvironment plays an important role in normal functioning of cells including proliferation, survival and production of ECM. We further examined the localization of cell adhesion and focal adhesion protein vinculin in cells encapsulated inside these hydrogels. As indicated in Fig. 6b, enhanced spreading and localized focal adhesion spots were observed in PEG/Gel/Silicate hydrogels compared to PEG only gels.

The fabrication of hydrogel microfibers does not appear to cause a decrease in cell survivability or proliferation. An important criteria for material selection was low viscosity. The precursor solutions were injectable even with a higher viscosity due to its shear-thinning characteristic. We believe the shear thinning effect was caused by the surface charge and alignment of exfoliated Silicate nanoparticles, enabling flow past each other.[24] This property was useful in encapsulating cells, engineering complex geometry and for minimally invasive therapies. In our initial mechanical studies, fibers were extruded in 10$\times$ PBS. The variable of osmotic shock was mitigated by extruded fibers in standard cell culture media. This procedure permitted immediate nutrient flow around the cells incorporated into the fiber. We believe this method is a viable method to include cells into hydrogels that can be later processed into complex architectures.

To demonstrate the capabilities of this system for engineering complex tissue, a proof-of-concept multicellular culture was investigated (Fig. 7). RFP mosJ cells were encapsulated within PEG/Gel/Silicates microfibers and GFP-3T3 cells were seeded on the microfiber surface. The presence of both Gel and Silicates enabled sufficient binding of GFP labeled preosteoblast cells to the surface of the fiber surrounding the encapsulated RFP mosJ cells. After 1 h, GFP-3T3 cells adhered on the surface of microfibers (Fig. 7b). The majority of adhered cells demonstrated spreading on the fiber surface within 24 h. The background in red

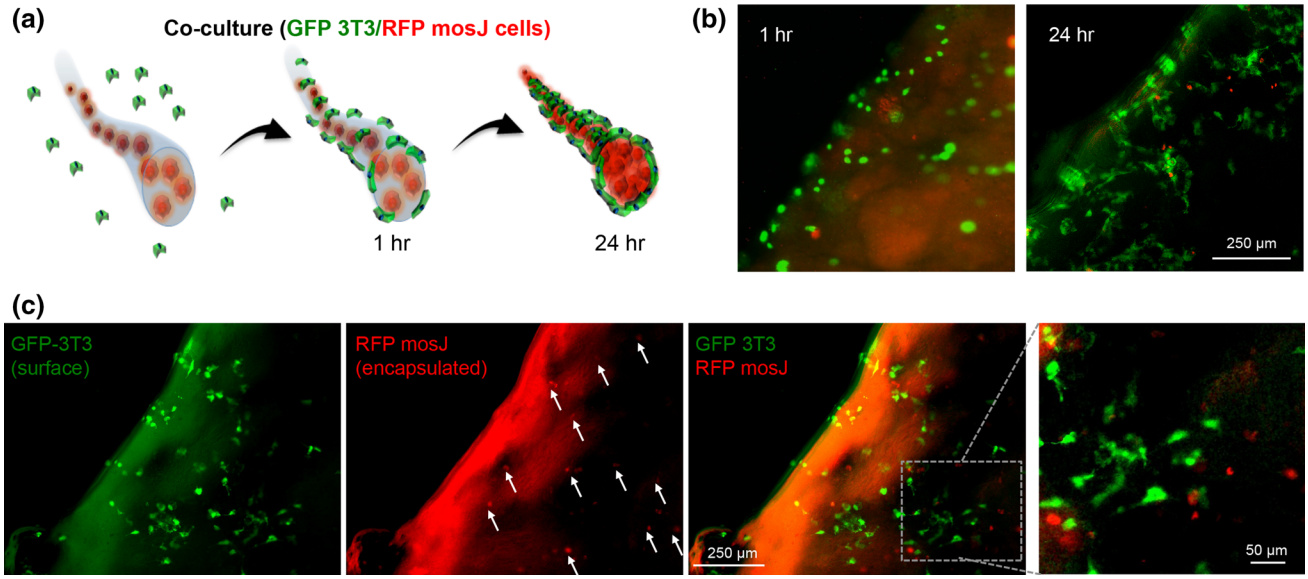

FIGURE 7. Engineering multicellular structures. (a) Co-culture of GFP-3T3 cells (green) on microfiber surface and RFP mosJ cells (red) inside the microfibers. (b) Morphology of cells after 1 and 24 h after seeding and encapsulation. RFP mosJ cell (red) inside the fiber remain circular, whereas seeded GFP 3T3 cells on surface readily spread. (c) Images showing presence of both the cells in microfibers, indicating their ability to form multicellular structures.

channel, make it difficult to locate the encapsulated cells. To visualize the RFP mosJ cells, high-resolution image of hydrogel microfiber was included (Fig. 7c). The arrow in the image indicates presence of encapsulated cells, which is evident in the inset. These results suggest that if let to proliferate, these cells would coat the surface and begin to form a stable shell around the secondary cells within.

CONCLUSION AND FUTURE DIRECTIONS

We demonstrate the fabrication of nanocomposite microfibers with tunable cell adhesion characteristic to engineer complex tissues structures. Spatiotemporal control over multiple cell types is needed and our system begins to address this challenge. The addition of Gel and Silicates to PEG render development of physically and covalently crosslinked network with tunable cellular characteristics by the incorporation of specific Gel amounts (cell adhesion) and the nature of the Silicates (mechanical stiffness and differentiation factors). Overall, the presented data suggest a promising future for the use of microfibers in cellular and drug delivery devices, and *ex vivo* cell culture models.

Further investigation is needed to understand the PEG/Gel/Silicates interactions during the polymerization. According to previously published reports, PEG chains can either interact with Silicate surface and/or form crosslinked network.[10] The addition of Gel appears to influence the mechanical properties; therefore, there must be a different interaction between PEG, Gel and Silicates. We hypothesize that the Gel backbone offers multiple binding sites for PEG and that the physical interactions between Silicate and PEG are more pronounced due to PEG chains being held in close contact to each other and the Silicates. Further studies must be completed to fully understand the interactions.

We foresee the potential for a variety of investigative directions with these materials.[5,22,40] The supplementation of bioactive natural and synthetic materials into the PEG matrix lends itself to the incorporation to a variety of biological agents, specifically those found in physiological environments with similar architectures. Endothelial cells provide a model of functional growth around a tubular polymer structure.[27,38] These endothelialized fibers could be subsequently arranged within a Gel network with a secondary cell type for more complex tissue organization. In a parallel study, the Silicates could be functionalized with a deliverable drug or protein, enabling controlled release from within the embedded fibers. This system could be utilized for a vascular diffusion model in which cells embedded within the Gel hydrogel could be tracked for migration kinetics or demonstrate spatiotemporal responses to the included agents within the fibers. Future studies will focus on tailoring the degradation of these fibers with the growth and formation of a functional cellularized conduit. Additionally, the shear-thinning characteristic of these composite hydrogels can be used for bioprinting, specifically to engineer complex tissue structures.

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CONFLICT OF INTEREST

Charles W. Peak, James K. Carrow, Ashish Thakur, Ankur Singh, and Akhilesh K. Gaharwar declare that they have no conflicts of interest.

ETHICAL STANDARDS

No animal or human studies were carried out by the authors for this article.

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