Published in final edited form as:

Biomacromolecules. 2011 May 9; 12(5): 1651–1657. doi:10.1021/bm200035r.

Injectable Multidomain Peptide Nanofiber Hydrogel as a Delivery Agent for Stem Cell Secretome

Erica L. Bakota[†], Yin Wang[‡], Farhad Danesh[‡], and Jeffrey D. Hartgerink^{†,§,*}

[†] Department of Chemistry, Rice University, 6100 South Main St., Houston, TX 77005

[§] Department of Bioengineering, Rice University, 6100 South Main St., Houston, TX 77005

[‡] Department of Nephrology, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030

Abstract

Peptide hydrogels show immense promise as therapeutic materials. Here we present a rationally designed multidomain peptide that self-assembles into nanofibers approximately 8 nm wide, 2 nanometers high and microns in length in the presence of Mg^{2+} . At a concentration of 1% by weight, the peptide forms an extensive nanofibers network that results in a physically cross-linked viscoelastic hydrogel. This hydrogel undergoes shear thinning and then quickly recovers nearly 100% of its elastic modulus when the shearing force is released, making it ideal for use as an injectable material. When placed in the presence of human embryonic stem cells (ESCs), the nanofibrous hydrogel acts like a sponge, soaking up the vast array of growth factors and cytokines released by the ESCs. The peptide hydrogel sponge can then be removed from the presence of the ESCs and placed in a therapeutic environment, where it can subsequently release these components. *In vitro* experiments demonstrate that release of stem cell secretome from these hydrogels in the presence of Glomerular Epithelial Cells treated with high glucose significantly decreased protein permeability in a model of diabetes-induced kidney injury. Tracking experiments were then performed to determine the fate of the hydrogel upon injection *in vivo*. Hydrogels labeled with a Gd^{3+} MRI contrast agent were injected into the abdominal cavity of mice and found to remain localized over twenty-four hours. This implies that the hydrogel possesses sufficient rigidity to remain localized and release stem cell secretome over time rather than immediately dissolving in the abdominal cavity. Together, the shear thinning and recovery as observed by rheometry, as well as secretome absorption and release *in vivo*, demonstrate the potential of the nanofibrous multidomain peptide hydrogel as an injectable delivery agent.

Keywords

hydrogel; self-assembly; peptide; injectable; embryonic stem cells

Introduction

Self-assembling peptide nanofiber hydrogels are a promising class of synthetic biomaterials that have been investigated for a wide variety of biomedical applications, including cell scaffolds^{1–6} and drug delivery agents.^{7–9} Controlled release from peptide hydrogels has been demonstrated with small molecules,^{9, 10} and with proteins, for example growth factors.^{11–13} Other hydrogel systems have also been demonstrated to deliver growth factors such as vascular endothelial growth factor (VEGF) to tissues.¹⁴ Peptide hydrogels have also been investigated as an injectable means of delivering stem cells.¹⁵ Stem cells are known to

have beneficial effects on tissues, derived from the wide array of compounds they secrete, through endocrine and paracrine effects.^{16–20} Any material with the ability to harness this secretome would carry enormous therapeutic potential. This concept has been demonstrated with peptide-based materials, in which nanofiber hydrogels were able to absorb and release proteins derived from stem cells and release them in a therapeutic setting.^{12, 21}

The powerful biomedical applications of peptide hydrogels derive from the ease with which new and diverse peptide sequences can be prepared, the nanostructure obtained through their self-assembly,²² the presentation of chemical information on their surface, and the rheological properties^{23–26} of the resulting hydrogel materials. Additionally, these self-assembling peptide nanofiber hydrogels can be designed to bind proteins, cells or to degrade in a predictable fashion with respect to time and location.

Recently we introduced a new class of self-assembling peptide nanofibers that we call multidomain peptides (MDPs).²² These peptides have an ABA block motif in which the central B block is composed of alternating hydrophilic and hydrophobic amino acids to create a facial amphiphile, while the peripheral A blocks are composed of charged amino acids. The role of the charged A block is to increase solubility and control self-assembly. Self-assembly in aqueous solution results in the formation of extended anti-parallel β -sheet nanofibers²² and, depending on the extent of assembly, results in the formation of a hydrogel.^{25, 27} Designed in this study is a new MDP, E₂(SL)₆E₂GRGDS (Figure 1), which incorporates the negatively charged glutamic acid in the A block and utilizes six pairs of alternating serine, leucine to create the amphiphilic B block. Additionally this peptide includes the well-known cell adhesion sequence RGD.^{28, 29} While nanofibers always form in water, gelation is triggered by a number of different methods that result in the neutralization or screening of the negative charges on the glutamic acid residues. A similar method of self-assembly has been used in other similar peptide systems.³⁰ While these salts are not required, they dramatically enhance the speed of gelation. In this study, MgCl₂ is used due to its compatibility with cell culture conditions.

Experimental

Peptide Synthesis and characterization

Multidomain peptides were synthesized by solid-phase peptide synthesis, as reported previously,²² with one modification: a Rink Amide MBHA low loading resin was used in place of a traditional Rink Amide MBHA resin. This resulted in higher yields than those obtained with standard resins. The N-terminus was acetylated with acetic anhydride prior to cleavage from the resin. E₂(SL)₆E₂GRGDS was purified by dialysis for 48 hours against deionized water in membranes with a 1,000 molecular weight cutoff. The solution was drained from the bags and lyophilized. Purified peptides were subsequently characterized by MALDI-TOF mass spectrometry. E₂(SL)₆E₂GRGDS, expected monoisotopic mass [M+H]⁺: 2249.1, Observed mass: 2249.6.

Peptide Gelation

A 1 wt% solution of E₂(SL)₆E₂GRGDS was prepared by dissolving the lyophilized powder in deionized water which was subsequently adjusted with NaOH to pH 7. While the peptide adopts a β -sheet structure in the absence of additional salts, the addition of MgCl₂ enhances nanofiber formation and results in the formation of a gel. Mg²⁺ to peptide ratios of 2:1 to 8:1 were investigated, and it was determined that the 4:1 ratio imparted the optimal rheological properties to the peptide hydrogel. MgCl₂ (1 M) at pH 7 was added so that the final ratio of Mg²⁺ to peptide was 4:1 (1% peptide by weight = 4.45 mM, thus Mg²⁺ is 17.8 mM). The vial was capped, shaken briefly, and sonicated to obtain a uniform gel, which formed within

seconds of mixing. Peptide preparations described as un-gelled are prepared as above but without addition of MgCl_2 .

Atomic Force Microscopy

1 wt% peptide solutions at pH 7 (both gelled and un-gelled) were diluted to a concentration of 0.01–0.05 wt% with ultra pure water. Approximately 5–7 μL of these solutions were dropped onto freshly cleaved mica while spinning on a Headway Research, Inc. Photo-resist spinner. The sample was rinsed with deionized water for 4–5 seconds and spun for an additional 10 minutes. AFM images were collected in air, at ambient temperature, on a Digital Instruments Nanoscope IIIa atomic force microscope in tapping mode. Gelled and un-gelled samples were prepared and imaged separately. Data were collected in height and amplitude channels. Height profiles were measured using Nanoscope software.

Vitreous Ice Cryo-Transmission Electron Microscopy (cryo-TEM) of Peptide Gels

Vitreous ice cryo-TEM samples were prepared using a controlled environment vitrification system (Vitrobot, FEI). 2.6 μL of 2 wt% peptide gel were pipetted onto a holey carbon grid (Quantifoil R1.2/1.3) and blotted with filter paper (Ted Pella, Inc.) for 1 second. The sample then was quickly plunged into liquid ethane. The sample was transferred from liquid ethane to liquid nitrogen for storage. The sample was then transferred to a Gatan cryoholder (Gatan626DH) and imaged on a JEOL 2010 TEM at 200.0 kV and -176°C , equipped with a Gatan CCD camera and Gatan DigitalMicrograph.

Scanning Electron Microscopy (SEM)

100 μL aliquots of each gel were prepared and placed in a 24 well plate. Gels were dehydrated in a series ethanol/water solutions progressing from 30% ethanol to 100% ethanol over the course of 24 hours. The dehydrated gels were critical point dried using an Electron Microscopy Sciences EMS 850 critical point drier. They were then affixed to SEM pucks using conductive carbon tape. The pucks were sputter coated with 10–15 nm gold using a CRC-150 sputter coater and imaged using an FEI Quanta 400 ESEM at 20.00 kV.

Circular Dichroism Spectroscopy

CD spectra were recorded using a Jasco-810 spectropolarimeter. For non-gelled peptides, samples at 1 wt% and pH 7 (adjusted with NaOH when necessary) were placed in a quartz cuvette with a path length of 0.001 cm. For samples gelled with Mg^{2+} , the resulting 1 wt% gel at pH 7 was carefully pipetted into a 0.001 cm path length quartz cuvette, making sure to avoid bubble formation. Spectra were recorded at room temperature from 250 to 180 nm, with a 0.2 nm data pitch and a scan rate of 50 nm/min. Milidegrees of rotation were converted to molar residual ellipticity (MRE).

Attenuated Total Reflectance Infrared Spectroscopy (ATR-IR)

5 μL of 1 wt% $\text{E}_2(\text{SL})_6\text{E}_2\text{GRGDS}$ gel (gelled by the addition of Mg^{2+} , as described above) at pH 7 were pipetted onto a “Golden Gate” diamond window and dried under nitrogen until a thin film of peptide was achieved. IR spectra (32 accumulations) were taken using a Jasco FT/IR-660 spectrometer.

Rheometry

Rheological measurements were taken using a TA Instruments AR-G2 rheometer. Approximately 20 μL of peptide solution were placed within a preset gap of 250 μm on an 8 mm steel parallel plate geometry. A frequency sweep was performed at 25°C and a frequency of 1 rad/sec was found to be in the linear viscoelastic region (LVR). This value was used to perform a strain sweep at 25°C and 1 rad/sec. Time sweep tests were performed

at 25 °C to determine the initial stability of the hydrogel as well as the recovery ability after shear. No breakdown of the gel was detected during time sweep measurements. In shear and recovery tests, the sample was pre-sheared to 100% strain for 1 minute and then a time sweep was performed at a frequency of 1 radian/sec and 0.5% strain for 20 minutes. To ensure that the sample only underwent shear rather than slipping, the phase angle was kept below 90°.

Gadolinium Labeling of Peptides

The protocol for gadolinium labeling was obtained from BioPal (BioPAL, Inc. Worcester, MA) and followed as written. Dry peptide was dissolved in 0.2 M carbonate buffer at pH 8 and set aside. Separately, 393 μ L of a solution of 1 M sodium acetate and 1 M NaOH were added to a 2 mL vial containing ~ 5 mg proprietary chelate. 7 μ L of 1 M GdCl₃ were then added to this solution, which was then capped and vortexed until all solids were dissolved. This solution was allowed to sit for 5 minutes, and then 3 equivalents of the Gd-chelate solution were added to the peptide solution. This solution was allowed to mix for 2 hours and was then purified by dialysis and subsequently lyophilized. The powder was reconstituted in phosphate-buffered saline and gelled by addition of Mg²⁺.

Animal Setup for MRI Tracking

Female C57BLKS/J mice weighing 20–30 g were purchased from Jackson Lab. Mice were intubated and inhalational isoflurane (1.5–2%) was administered through the endotracheal tube. Pulse and oxygenation, temperature, end-tidal CO₂ and respiratory rate were monitored during the entire MRI scan period. The mice were kept warm using a water heater. 200–300 μ L Gd-conjugated nanofibers were injected into the mice using a 1 mL syringe with a 23 gauge needle.

MRI Tracking Experiment

Experiments were performed using a vertical bore 11.7 T Bruker Avance imaging spectrometer with a microimaging gradient insert and 30 mm birdcage RF coil (Bruker Instruments, Billerica, MA). T1-weighted images were acquired using a 2D gradient echo sequence. The sequence parameters were: TR = 500ms and TE = 8.233ms, Field of View (FOV) 40 $\times$ 40mm, Matrix size 256 $\times$ 256, 30 consecutive coronal slice with slice thickness 0.5 mm. 4 averages were taken, with a scan time of 6 minutes, 24 seconds. To minimize motion artifacts, data acquisition was gated (i.e. synchronized to the respiratory signal). After injection, scans were acquired at 30 mins, 1.5 hrs, 3 hrs, 6 hrs, 12 hrs and 24 hrs. A control experiment was performed, in which PBS was injected with no peptide nanofibers. A total of 5 mice were used for this experiment.

BSA clearance experiments, Cell culture

Conditionally immortalized renal microvascular endothelial cells were kindly provided by Dr. Robert Langley (The University of Texas M. D. Anderson Cancer Center). Human ES cell line H9 was obtained from WiCell Research Institute (Madison, WI). H9 cells were plated on irradiated (35 gray g irradiation) mouse embryonic fibroblasts. Culture medium for the present work consisted of 80% DMEM-F12 media (Gibco BRL, Rockville, MD), 1 mM L-glutamine, 0.1 mM β -mercaptoethanol, 1% nonessential amino acids stock (Gibco BRL) and 4 ng/mL human basic fibroblast growth factor (bFGF: Invitrogen), supplemented with 20% KnockOut SR, a serum replacer optimized for mouse ES cells (Gibco BRL). Routine passages of hES-H9 cells were done every 5–7 days with collagenase (type IV, 1 mg/mL, Invitrogen) treatment and mechanical scraping. The ES cells were maintained on irradiated mouse embryonic fibroblasts (MEFs) at a density of 19,500 cells/cm² in 6-well culture plates (Nunc) as previously described.²¹ In brief, ES cells were maintained in DMEM/F12

culture medium supplemented with 20% KnockOut serum replacer, 0.1 mmol/L nonessential amino acids, 1 mmol/L L-glutamine (all from Invitrogen), and 0.1 mmol/L mercaptoethanol (Sigma). In addition, the medium was supplemented with 4 ng/mL human recombinant basic fibroblast growth factor (Invitrogen). Routine passages of hES-H9 cells every 5–7 days were done with collagenase (type IV, 1 mg/mL, Invitrogen) treatment and mechanical scrape.

Preparation of “preconditioned” nanofibers

Nanofibers were preconditioned with hES cells by using two-compartment transwell co-culture system (6-well plates) (BD Biocoat, MA), in which hES were seeded in the upper compartment, and nanofibers were placed in the lower compartment in the presence of hES cell medium without KnockOut SR (serum replacement). After 24 hrs of conditioning, nanofibers were removed and collected for permeability experiments. This culture setup has been described previously in more detail.²¹

Transendothelial ¹²⁵I-BSA permeability measurements

A Two-compartment transwell co-culture system (24-well plates)(BD) was used to determine the permeability of ¹²⁵I-BSA (bovine serum albumin) across confluent endothelial cell monolayers as previously described. Clearance rate of ¹²⁵I-BSA was calculated from the formula: $CL(\mu\text{L}/\text{min}) = [\text{activity}_{AC}(\text{cpm}/\mu\text{L}) \times \text{volume}_{AC}(\mu\text{L})] / [\text{activity}_{LC}(\text{cpm}/\mu\text{L}) \times \text{time}(\text{min})]$, in which CL is clearance rate (volume of luminal chamber fluid cleared of tracer), activity_{AC} is counts per mL of abluminal chamber sampling, volume_{AC} is total abluminal chamber volume at the time of sampling, and activity_{LC} is counts per μL of luminal chamber fluid added at time 0. Endothelial cells were cultured in the presence of endothelial cell medium. For statistical analysis, ANOVA was performed using Graphpad software. Error bars are $\pm$ standard error of the mean (SEM) and asterisks indicate as follows: * $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$

Ethical Guidelines

All local and ethical guidelines of Rice University and Baylor College of Medicine were followed in the course of these experiments. Stem cells were transferred per agreement with Wisconsin Materials. Additionally, no materials involved in this study were used for commercial purposes.

E₂(SL)₆E₂GRGDS was prepared via solid phase peptide synthesis and verified using MALDI-TOF mass spectrometry. After dialysis against deionized water, this peptide was characterized using circular dichroism (CD) and infrared (IR) spectroscopy. CD spectra indicate a β -sheet secondary structure, with minima at 217 nm and maxima at 197 nm (Figure 2a). Gelation with Mg²⁺ reinforced this signal, inducing an increase in the maximum at 197 nm with a simultaneous decrease at 217 nm. The IR spectra indicate the presence of an anti-parallel β -sheet structure with an amide I_{||} band at 1616 cm⁻¹ and amide I_⊥ at 1690 cm⁻¹ (Figure 2b). IR spectra prior to gelation show identical features to the hydrogel (data not shown). Atomic force microscopy (AFM, Figure 3) and vitreous ice cryo-transmission electron microscopy (TEM, Figure 4a and 4b) confirmed the presence of an extended nanofiber network. These peptide nanofibers result in the formation of a hydrogel in the presence of cell culture media that has been supplemented with Mg²⁺ (Figure 4c). It has been well-established that ion screening of charged amino acids plays a huge role in the formation of self-assembled peptide nanofibers.³⁰ This hydrogel can be visualized by critical point-drying, followed by SEM, which reveals a sponge-like internal structure (Figure 4d).

Previous MDPs have demonstrated cytocompatibility,²⁷ making them appealing for use in biomedical applications such as a drug delivery vehicle *in vitro* and *in vivo*. Useful

properties for a peptide drug delivery hydrogel include: stability of the hydrogel over time, the ability to undergo shear thinning and subsequently recover, and the ability of the hydrogel to be loaded with therapeutics and subsequently release them in a targeted environment. The multidomain peptide E₂(SL)₆E₂GRGDS satisfies all of these conditions. The stability of this peptide was first tested on a controlled-stress rheometer by performing a time sweep, which showed that the peptide hydrogel was stable over the time frame necessary to complete rheological tests. The peptide hydrogel was also measured over the course of several weeks, and no significant changes were observed. Thus, incubation time does not substantially effect the rheological properties of the hydrogel. The rigidity of this peptide was tested on a controlled-stress rheometer by performing a strain sweep (Figure 5a), which shows a storage modulus of approximately 480 Pa. One potential limitation of peptide hydrogels is that their fragility may make delivery of the hydrogel in a biological setting impractical. On the other hand, excessive robustness without shear thinning may preclude injection of a hydrogel altogether. Peptide materials have been shown to avoid both of these issues, making them ideal drug delivery systems.⁹ E₂(SL)₆E₂GRGDS specifically demonstrates an ability to both undergo shear thinning and recover rapidly after needle shear, thus allowing injection of the peptide hydrogel directly into the site of interest without permanent loss of hydrogel properties. Needle shear was simulated by applying 100% strain for 1 minute. This was immediately followed by monitoring the recovery of the elastic modulus, G', over time. It was found that this peptide recovers over 75% of its elastic modulus immediately (within 13 seconds, the smallest time interval measured during rheometry) and 100% of its elastic modulus within 10 minutes of removal of the shear (Figure 5b). However, it is important to note that simulated needle shear may not be identical to actual needle shear due to differences in geometry between the rheometer and a syringe, so shear-thinning tests were then performed using a syringe and 21 G needle. In these experiments, the peptide was placed carefully on the rheometer plate (taking care to disturb the gel as little as possible) and a time sweep was performed to measure the elastic modulus of the gel over 15 minutes. Then the peptide was removed, loaded with a spatula into a syringe fitted with a 21 G needle, and the gel was squirted through the needle onto the rheometer plate, where the elastic modulus of the gel was monitored over another 15-minute interval (Figure 6). After shearing through the syringe and needle, it was evident that the G' of the gel was not affected. Thus, gels of this type are well-suited for delivery via injection.²⁶

The peptide hydrogel's ability to be used as a drug delivery matrix was then examined. First, a tracking experiment was performed to ascertain the fate of the hydrogel upon injection. Gadolinium is frequently used to enhance contrast in magnetic resonance imaging (MRI), and it is estimated that at least 30% of MRIs performed today utilize chelated gadolinium for this purpose.³¹ A Gd³⁺ labeled version (see Supporting Information) of the hydrogel was prepared and carefully injected into the abdominal cavity of a mouse, and the localization of the hydrogel was monitored by magnetic resonance imaging (MRI). After injection, scans were acquired at 30 minutes, 1.5 hours, 3 hours, 6 hours, 12 hours and 24 hours. All experiments were repeated at least in triplicate.^{32–35} Figure 7 shows that the labeled hydrogel can be visualized at 24 hours after injection and demonstrates that a peptide hydrogel could be delivered to a site of interest without immediate dissolution into the surrounding body tissues. This lack of dissolution is related to the ability of this hydrogel to recover after shearing through the syringe and needle. The rapid recovery of the elastic modulus after needle shear allows the gel to regain its nanostructure faster than the time that is required to flow into the surrounding body tissues.

It has been shown that both bone marrow-derived stem cells and mesenchymal stem cells (MSCs) can accelerate tissue repair and healing after renal injury.^{36–40} While the use of embryonic stem cells has shown great promise to aid in healing a large variety of diseases

and injury, the use of embryonic stem cells carries with it a variety of risks including immune rejection and the formation of teratomas.⁴¹ Thus, an acellular approach to stem cell therapies is desired. Additionally, the actual mechanism of the beneficial effects of stem cells is not entirely clear. It has been suggested that rather than repairing damaged tissue directly, the stem cells rely on endocrine and paracrine effects, secreting a wide variety of cytokines, chemokines, and growth factors, which aid the existing tissue's ability to repair itself.^{16–20} The full register of cytokines and growth factors secreted by ESCs is still unknown and is currently being investigated. Prior work has shown that this register of cytokines and growth factors is composed of at least 36 secreted proteins, including but not limited to osteopontin, follistatin, ICAM-5, IL-12 p40/p70, CCR10 and adiponectin.²¹ However, this suggests that it is the cytokines and growth factors that are required in a potential therapy, not necessarily the cells themselves. In fact, cell culture medium that has been preconditioned in the presence of MSCs has been shown to enhance endothelial cell proliferation and differentiation.²⁰ While the use of preconditioned medium eliminates the need for direct injection of cells, the coupling of preconditioned medium with a dense nanofibers matrix may increase the effect of these cytokines and chemokines and possibly extend their duration of effect. Utilizing this concept we “preconditioned” the multidomain peptide nanofiber hydrogel by incubating it in the presence of embryonic stem cells (Figure 8).

The hydrogel acts as a sponge, soaking up the stem cell secretome while being separated from the stem cells themselves via a permeable membrane. During incubation, the peptide gel is not in contact with the ESCs, but the permeable membrane allows stem cell secretome to diffuse through the culture medium and into the gel. After this preconditioning step, the hydrogel can be used in a cell free manner to treat the condition of interest. A similar approach was recently used for the treatment of cardiovascular disease.¹² In our work we test the ability of preconditioned multidomain peptide nanofiber hydrogels to ameliorate a cell culture model for diabetes induced kidney damage. Glomerular Epithelial Cells (GECs) in the kidney form a selectively permeable membrane, which becomes increasingly leaky to proteins at high concentrations of glucose. After the preconditioning step, the hydrogel is placed in a culture of Glomerular Epithelial Cells (GECs) and the permeability of I¹²⁵ BSA is monitored over time under both low and high glucose conditions. The low glucose condition serves as a control, showing that cell permeability is not increased under these conditions. As an additional control, hydrogels “preconditioned” by the ESC fibroblast feeder layer were used (pre-MEF-NF). In this control, only the fibroblast layer was used; no ESCs were present. As expected, GECs were found to have increased permeability in the presence of high glucose levels, which is observed by the 50% increase in clearance rate of I¹²⁵ BSA (Figure 9). It was found that in the presence of ESC secretome-loaded MDP hydrogels (pre-hES-NF), the permeability of GECs was reduced to approximately the level of cells treated with low glucose. In contrast, MDP hydrogels preconditioned with the mouse fibroblast feeder layer had no significant effect on BSA clearance.

In conclusion, we have shown that the multidomain peptide hydrogel self-assembled from E₂(SL)₆E₂GRGDS is a suitable biomaterial for ESC secretome delivery. It forms a stable, nanofibrous hydrogel in the presence of Mg²⁺ and undergoes rapid shear thinning and recovery, allowing it to be easily delivered by syringe. Labeling with gadolinium via a covalently attached chelate illustrates the localization of the β -sheet nanofiber network after injection *in vivo*. Drug loading and delivery is demonstrated by preconditioning the hydrogel in the presence of ESC and using these cell-free constructs to remediate a model of diabetes. These nanofibers constructs release at least 36 different secreted proteins, although the full extent of compounds absorbed and released by the peptide hydrogel is still being investigated.²¹ The hydrogels preconditioned with these compounds nevertheless showed a significant effect on BSA clearance in an *in vitro* model. Similarly encouraging results have

recently been obtained *in vivo* with peptide-based nanomaterials.¹² Other release studies using RADA 16 have shown that some molecules interact more strongly with peptide nanofibers than others.⁹ Thus, some components of stem cell secretome may have been partially retained in the hydrogel scaffold, while other components may have been released completely.

Acknowledgments

This work was funded in part by the Robert A. Welch Foundation (C1557), the NSF (DMR-0645474) the NIH (DK067604 and DK078900) and the Camille Dreyfus Teacher Scholar Awards Program.

References

1. Hule RA, Nagarkar RP, Altunbas A, Ramay HR, Branco MC, Schneider JP, Pochan DJ. *Faraday Discuss.* 2008; 139:251–264.
2. Zhang S. *Nat Biotechnol.* 2003; 21:1171–1178. [PubMed: 14520402]
3. Ellis-Behnke RG, Liang YX, You SW, Tay DK, Zhang S, So KF, Schneider GE. *Proc Nat Acad Sci U S A.* 2006; 103:7530.
4. Jung JP, Jones JL, Cronier SA, Collier JH. *Biomaterials.* 2008; 29:2143–2151. [PubMed: 18261790]
5. Kisiday J, Jin M, Kurz B, Hung H, Semino C, Zhang S, Grodzinsky AJ. *P Nat Acad Sci U S A.* 2002; 99:9996–10001.
6. Holmes TC, de LS, Su X, Liu GS, Rich A, Zhang SG. *P Nat Acad Sci U S A.* 2000; 97:6728–6733.
7. Branco MC, Schneider JP. *Acta Biomaterialia.* 2009; 5:817–831. [PubMed: 19010748]
8. Branco MC, Pochan DJ, Wagner NJ, Schneider JP. *Biomaterials.* 2009; 30:1339–1347. [PubMed: 19100615]
9. Nagai Y, Unsworth LD, Koutsopoulos S, Zhang S. *J Controlled Release.* 2006; 115:18–25.
10. Naskar J, Palui G, Banerjee A. *J Phys Chem B.* 2009; 113:11787–11792. [PubMed: 19708711]
11. Koutsopoulos S, Unsworth LD, Nagaia Y, Zhang SG. *P Nat Acad Sci U S A.* 2009; 106:4623–4628.
12. Webber MJ, Han X, Murthy SN, Rajangam K, Stupp SI, Lomasney JW. *J Tissue Eng Regen M.* 2010; 4:600–610. [PubMed: 20222010]
13. Gelain F, Unsworth LD, Zhang SG. *J Controlled Release.* 2010; 145:231–239.
14. Emerich DF, Mooney DJ, Storrie H, Babu RS, Kordower JH. *Neurotox Res.* 2010; 17:66–74. [PubMed: 19588214]
15. Haines-Butterick LA, Rajagopal K, Branco MC. *Proc Nat Acad Sci U S A.* 2007; 104:7791–7796.
16. Bi B, Schmitt R, Israilova M, Nishio H, Cantley LG. *J Am Soc Nephrol.* 2007; 18:2486–2496. [PubMed: 17656474]
17. Imberti B, Morigi M, Tomasoni S, Rota C, Corna D, Longaretti L, Rottoli D, Valsecchi F, Benigni A, Wang J, Abbate M, Zoja C, Remuzzi G. *J Am Soc Nephrol.* 2007; 18:2921–2928. [PubMed: 17942965]
18. Togel F, Hu ZM, Weiss K, Isaac J, Lange C, Westenfelder C. *Am J Physiol-renal.* 2005; 289:F31–F42.
19. Togel F, Isaac J, Hu ZM, Weiss K, Westenfelder C. *Kidney Int.* 2005; 67:1772–1784. [PubMed: 15840024]
20. Togel F, Weiss K, Yang Y, Hu ZM, Zhang P, Westenfelder C. *Am J Physiol-renal.* 2007; 292:F1626–F1635.
21. Wang Y, Bakota E, Chang BHJ, Entman M, Hartgerink JD, Danesh FR. *J Am Soc Nephrol.* 2011 doi: 10.1681.
22. Dong H, Paramonov SE, Aulisa L, Bakota EL, Hartgerink JD. *J Am Chem Soc.* 2007; 129:12468–12472. [PubMed: 17894489]
23. Rajagopal K, Lamm MS, Haines-Butterick LA, Pochan DJ, Schneider JP. *Biomacromolecules.* 2009; 10:2619–2625.

24. Branco MC, Nettesheim F, Pochan DJ, Schneider JP, Wagner NJ. *Biomacromolecules*. 2009; 10:1374–1380.
25. Aulisa L, Dong H, Hartergink JD. *Biomacromolecules*. 2009; 10:2694–2698.
26. Ramachandran S, Tseng Y, Yu YB. *Biomacromolecules*. 2005; 6:1316–1321.
27. Galler KM, Aulisa L, Regan KR, D'Souza RN, Hartgerink JD. *J Am Chem Soc*. 2010; 132:3217–3223. [PubMed: 20158218]
28. Ruoslahti E, Pierschbacher MD. *Science*. 1987; 238:491–497. [PubMed: 2821619]
29. Burdick JA, Anseth KS. *Biomaterials*. 2002; 23:4315–4323. [PubMed: 12219821]
30. Zou DW, Tie ZX, Lu CM, Qin M, Lu XM, Wang M, Wang W, Chen P. *Biopolymers*. 2010; 93:318–329. [PubMed: 19885921]
31. Caravan P, Ellison JJ, McMurry TJ, Lauffer RB. *Chem Rev*. 1999; 99:2293–2352. [PubMed: 11749483]
32. Pautler RG. *Physiology*. 2004; 19:168–175. [PubMed: 15304630]
33. Pautler RG, Mongeau R, Jacobs RE. *Magnet Reson Med*. 2003; 50:33–39.
34. Kim SH, Csaky KG, Wang NS, Lutz RJ. *Pharm Res*. 2008; 25:512–520. [PubMed: 17674155]
35. Chen XM, Astarly GW, Sepulveda H, Mareci TH, Sarntinoranont M. *Magn Reson Imaging*. 2008; 26:1433–1441. [PubMed: 18583082]
36. Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G. *Int J Mol Med*. 2004; 14:1035–1041. [PubMed: 15547670]
37. Humphreys BD, Bonventre JV. *Annu Rev Med*. 2008; 59:311–325. [PubMed: 17914926]
38. Ikarashi K, Li B, Suwa M, Kawamura K, Morioka T, Yao J, Khan F, Uchiyama M, Oite T. *Kidney Int*. 2005; 67:1925–1933. [PubMed: 15840040]
39. Kale S, Karihaloo A, Clark PR, Kashgarian M, Krause DS, Cantley LG. *J Clin Invest*. 2003; 112:42–49. [PubMed: 12824456]
40. Lin FM, Cordes K, Li LH, Hood L, Couser WG, Shankland SJ, Igarashi P. *J Am Soc Nephrol*. 2003; 14:1188–1199. [PubMed: 12707389]
41. Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. *Nat Biotechnol*. 2000; 18:399–404. [PubMed: 10748519]

Figure 1. Self-assembly of $E_2(SL)_6E_2GRGDS$ into nanofibers. (a) Chemical structure of $E_2(SL)_6E_2GRGDS$. (b) Scheme depicting assembly of the peptide dimer repeating unit, a “hydrophobic sandwich” that assembles into a nanofiber.

Figure 2.

(a) Circular dichroism (CD) measurements of E₂(SL)₆E₂GRGDS before and after gelation with Mg²⁺. (b) Infrared (IR) spectroscopy of E₂(SL)₆E₂GRGDS after gelation.

Figure 3. Atomic force microscopy (AFM) of $E_2(SL)_6E_2GRGDS$ (a) before gelation and (b) after gelation with Mg^{2+} . White lines in left images indicate the axis of height profiles shown at right. Red triangles denote the points between which the height differential was measured. Images are $3 \times 3 \mu m$.

Figure 4.
(a & b) Vitreous ice cryo-TEM of $E_2(SL)_6E_2GRGDS$ nanofibers, (c) a hydrogel of nanofibers formed in cell culture medium, (d) SEM of the resulting peptide hydrogel.

Figure 5.

(a) Strain sweep performed after gelation with Mg^{2+} . (b) Time sweep performed on $E_2(SL)_6E_2GRGDS$ gel, with shear being applied at $T = -1$ and removed at $t=0$.

Figure 6. Time sweeps performed on the peptide hydrogel before needle shear (red) and after needle shear (blue) using a syringe fitted with a 21 G needle.

Figure 7.

Left: Nanofibrous MDP hydrogel undergoes shear thinning and shear recovery allowing simple injection in vivo. *Right:* MRI tracking of gadolinium-labeled nanofibers 24 hours after injection into the abdominal cavity of mouse. The red arrow indicates the position of the localized hydrogel.

Figure 8.
Schematic representation of the two-compartment transwell coculture system used to precondition peptide nanofibers.

Figure 9.

Relative permeability rate of albumin of glomerular epithelial cells incubated in low (LG) and high glucose (HG) conditions. Cells in high glucose conditions were also incubated with MDP hydrogel that had been preconditioned either with human embryonic stem cells (pre-hES-NF) or with the mouse fibroblast feeder layer (pre-MEF-NF). Data are shown as mean $\pm$ SEM. Statistical significance was assessed by performing analysis of variance (ANOVA) followed by the Tukey-Kramer post-hoc analysis for multiple comparisons using an alpha value of 0.05. * $P < 0.05$, ** $P < 0.01$.