Published in final edited form as:
J Am Chem Soc. 2010 March 10; 132(9): 3217–3223. doi:10.1021/ja910481t.

Self-assembling Multidomain Peptide Hydrogels: Designed Susceptibility to Enzymatic Cleavage Allows Enhanced Cell Migration and Spreading

Kerstin M. Galler[1,2], **Lorenzo Aulisa**[3], **Katherine R. Regan**[3], **Rena N. D'Souza**[4], and **Jeffrey D. Hartgerink**[1,3,*]

[1] Department of Bioengineering, Rice University, Houston, TX

[2] Department of Restorative Dentistry, University of Regensburg, Germany

[3] Department of Chemistry, Bioengineering, Rice University, Houston, TX

[4] Department of Biomedical Sciences, Baylor College of Dentistry, Texas A&M, Dallas, TX

Abstract

Multidomain Peptides are a class of amphiphilic self-assembling peptides with a modular ABA block motif in which the amphiphilic B block drives self-assembly while the flanking A blocks, which are electrostaticly charged, control the conditions under which assembly takes place. Previously we have shown that careful selection of the amino acids in the A and B blocks allow one to control the self-assembled fiber length and viscoelastic properties of formed hydrogels. Here we demonstrate how the modular nature of this peptide assembler can be designed for biological applications. With control over fiber length and diameter, gelation conditions and viscoelastic properties, we can develop suitable materials for biological applications. Going beyond a simple carrier for cell delivery, a biofunctional scaffold will interact with the cells it carries promoting advantageous cell-matrix interactions. We demonstrate the design of a multidomain peptide into a bioactive variant by incorporation of a matrix metalloprotease-2 (MMP-2) specific cleavage site and cell adhesion motif. Gel formation and rheological properties were assessed and compared to related peptide hydrogels. Proteolytic degradation by collagenase IV was observed in a gel weight loss study, and confirmed by specific MMP-2 degradation monitored by mass spectrometry and cryo-TEM. Combination of this cleavage site with the cell adhesion motif RGD resulted in increased cell viability, cell spreading, and encouraged cell migration into the hydrogel matrix. Collectively the structural, mechanical and bioactive properties of this multidomain peptide hydrogel make it suitable as an injectable material for a variety of tissue engineering applications.

Introduction

Hydrogels have great potential for numerous tissue engineering and drug delivery applications due to their high water content, tissue-like viscoelastic properties and diffusion characteristics. They are easy to handle, injectable, and cells can be encapsulated and evenly distributed in the hydrogel matrix. Natural materials such as collagen, alginate, and fibrin

*Corresponding author: Jeffrey D. Hartgerink, PhD, Associate Professor, Departments of Chemistry and Bioengineering, Rice University, Houston, TX 77251-1892, Office: 713-348-4142, jdh@rice.edu.

Supporting Information Available: additional CD and MALDI-TOF MS analysis. This information is available free of charge via the Internet at http://pubs.acs.org/.

have been used for cell or drug delivery, but limited control over the gelation process, mechanical properties and degradation kinetics have led to modifications, combinations and the development of synthetic hydrogels.[1] Whereas previously, these materials were utilized as bioinert, passive vehicles to temporarily provide structural support to cells, hydrogels are now used to create bioactive materials which display biological cues that promote specific cell-matrix interactions. The overarching goal is to generate scaffolds with close structural and biochemical similarity to the cells' natural environment, the extracellular matrix (ECM).

The ECM mainly consists of a fibrous network of collagen, which provides tensile strength and support to cells. Elastin enables stretch and recoil, and proteoglycans bind water, supply compression resistance and sequester growth factors. Various non-collagenous proteins orchestrate the cells' dynamic behavior such as adhesion, migration and differentiation. The cells receive cues from the ECM, but at the same time, they actively secrete, interact with and constantly remodel their environment, thus re-enforcing their phenotype. By excreting proteases, mainly plasmin or matrix metalloproteinases (MMPs), cells can degrade the matrix around them and replace it with newly synthesized ECM proteins. This process in turn affects cellular behavior as it has been found that MMPs are associated with proliferation, migration, apoptosis and differentiation during development, tissue remodeling and angiogenesis. However, MMPs also play a role in pathological processes and cancer formation.[2] The MMP-2 gene encodes an enzyme which degrades collagen IV, the type of collagen present in basement membrane and also has the ability to degrade several other types of collagen including fibrillar collagen type I, the main component in extracellular matrix of connective tissues.[3]

Although ECM mimics which reproduce all ECM characteristics have yet to be developed, a number of attractive materials with key ECM properties have been described. [4-13] Recently, our laboratory has developed an amphiphilic, self-assembling peptide system we call multidomain peptides (MDP) due to their modular design.[14, 15] The MDPs are designed to display distinct regions of function or “domains” arranged in an ABA block motif, and self-assemble into nanofibers 6 nm in diameter with design dependant length. The process of supramolecular assembly is driven by a core motif (the B block) of alternating hydrophilic and hydrophobic amino acid residues. In an aqueous environment, the side chains segregate to opposing sides of the peptide backbone creating a facial amphiphile. Two of these peptides can then form a “sandwich” stabilized by hydrophobic packing between leucine residues. Fiber elongation occurs as the dimers string together through anti-parallel β-sheet hydrogen bonding oriented down the fiber axis. Charged amino acid residues in the flanking **A** region provide water solubility and also work against fiber assembly via electrostatic repulsion and can thus be utilized to control fiber assembly and length.[14] For example, addition of oppositely charged multivalent ions can screen these charges and result in physical crosslinking, fiber elongation, entrapment of water and gelation. [15]

With a fiber diameter of 6 nm, nanofibers created from MDPs mimic the nanoscale dimensions and structure of natural ECM, where cells can bind to the fibers via adhesion molecules, but still interact with other cells.[16] True three-dimensional cell growth within the nanofibrous gels make these materials promising candidates as scaffolds for cell delivery. Incorporation of bioactive sequences in either the central block or the flanking regions of MDPs may enhance cell-matrix interactions and promote desired cellular responses. However, the organization of MDP molecules into organized β-sheet aggregates with a fiber assembly process and structure reminiscent of amyloid raises concerns that the material may not be degradable *in vivo* and therefore might not be suitable as a tissue engineering scaffold. This is underscored by the devastating effects of amyloid aggregates observed in Alzheimer's disease. By programming susceptibility to proteolytic degradation into our β-sheet forming peptides, MDPs may be adapted to a variety of biological applications. Based

on the general ABA block structure of multidomain peptides as exemplified in $K_2(SL)_6K_2$ (**MDP1**, see table 1),[15] a hexapeptide containing the MMP-2 consensus cleavage motif LRG[17] in the central block motif was designed. We show that this modification results in susceptibility to degradation with collagenase IV and, to a lesser extent, trypsin, by gel weight loss over time. Additionally, specific degradation with MMP-2 was observed by mass spectrometry and cryo-TEM. Incorporation of both the cleavage site and the well known three amino acid cell adhesion motif "RGD"[18] made this variant compatible for cell culture leading to good cell spreading, proliferation and increased migration into the assembled hydrogel.

Experimental

Peptide synthesis

All peptides were synthesized by solid phase chemistry on an Advanced Chemtech Apex 396 peptide synthesizer using a protocol as described previously[15] which had been optimized for strongly β-sheet forming peptides. After acylation of the N-termini and cleavage from the resin, the crude peptides were dissolved in de-ionized water at 5 mg/mL and dialyzed in semipermeable membranes with a molecular weight cut-off of 100-500 Da (Spectra/Por, Spectrum Laboratories Inc., Rancho Dominguez, CA). The water was changed every 12 hours for 5 days, the peptides were lyophilized and the resulting products were used for further analysis and cell culture experiments. The correct masses of all peptides were confirmed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopy (see supporting information).

Gel formation and rheological properties

The lyophilized peptides were dissolved in de-ionized water containing 298 mM sucrose at 20 mg/mL, and the pH was adjusted to 7.4. Gelation was induced by addition of phosphate buffer where lysine-containing peptides are cross-linked due to the presence of negatively charged phosphate ions, to a final peptide concentration of 10 mg/mL (1% by weight). To evaluate viscoelasticity and gelation behaviors of MDP hydrogels, oscillatory stress sweep analysis was performed 24 hours after induction of gelation (AR-G2, TA Instruments, 8mm parallel steel-plates). 50 μL of gel were pipetted onto the center of the plate and a gap of 250 μm was established. Storage modulus (G′) and loss modulus (G″) were measured as a function of oscillatory stress ranging from 0.01 to 1000 Pa at an angular frequency 0.5 rad/s.

Weight Loss

To determine the degradation profile of MDP's with and without an MMP-2 cleavage sequence **MDP1** and **4** hydrogels were prepared as described above, and for each sample, 50μL of gel were transferred to a centrifuge tube after weighing the empty tube. After 30 min of equilibration, 200 μL PBS was added on top of each gel, and samples were stored at 37°C for 2 hrs. The PBS solution was then removed, and the weight of each gel was determined. Samples were divided into 3 groups and incubated with 200 μL of 1) collagenase IV (Sigma-Aldrich, St. Louis, MO) at 3mg/mL in PBS, 2) trypsin 0.05% with EDTA 4Na (Invitrogen, Carlsbad, CA), or 3) PBS as a negative control. Samples were run in triplicates, solutions were changed and the sample weight was determined every 24 hrs for 14 days.

MMP-2 specific cleavage

To assess MMP-2 specific cleavage, gels of **MDP1** and **4** and **5** were prepared at a concentration of 20 mg/mL, and then mixed with MMP-2 (Sigma-Aldrich, St. Louis, MO) in PBS, with the final concentrations being 10 mg/mL peptide and 100 ng MMP-2. Samples were incubated at 37°C for 48 hrs, and enzymatic cleavage was assessed by mass

spectroscopy. For **MDP4**, digestion was also visualized by vitreous ice cryo-TEM. For cryo-TEM analysis, a small quantity of the sample solution (2-3 μl) was applied to a TEM copper grid with holey carbon film purchased from Quantifoil (400 mesh Cu grid, 1.2 μm hole diameter), and blotted with filter paper using a Vitrobot type FP 5350/60 under 100% relative humidity for two seconds to create a thin layer of sample on the surface of the grid. The grid was plunged into liquid ethane and quickly transferred to liquid nitrogen. Samples were analyzed using JEOL 2010 TEM at an accelerating voltage of 200 kV under low-dose imaging conditions.

Cell proliferation and migration

To assess the compatibility of the newly created peptide hydrogel with cell cultures, **MDP1** and the peptide containing the cleavage sequence, **MDP4**, were modified to include the cell adhesion motif RGDS at the C-terminus, resulting in **MDP2** and **MDP5**. For cell viability and migration studies, mesenchymal stem cells from human exfoliated deciduous teeth (SHED) were used[19], which were kindly provided by Dr. Songtao Shi at USC. The cells were cultured in alpha MEM supplemented with 15% fetal bovine serum, 50 μg/mL L-ascorbic acid 2-phosphate, 100 U/mL penicillin and 100 μg/mL streptomycin, and incubated at 37°C with 5% CO_2. Subconfluent cells of passage 5 were detached using trypsin-EDTA (Invitrogen, Carlsbad, CA), and cells were encapsulated at a density of 1.0×10^5 per gel. Hydrogels were prepared at 20 mg/mL, and mixed with a cell suspension in PBS to create gels with a final peptide concentration of 10 mg/mL. Gels containing SHED were seeded in 96-well plates with a gel volume of 100 μL, and 200 μL of medium was added on top of each gel 30 min later. Culture medium was changed every other day, and MTT assay for cell viability was performed after 3, 7 and 14 days of incubation as follows: Gels were incubated with 200 μL of medium without serum containing 2 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, St. Louis, MO) for 3 hours. The solution was removed, and gel and cells were lysed in 200 μL DMSO (Sigma-Aldrich, St. Louis, MO), the plates were shaken thoroughly for 5 min. Absorbance was measured in a 96-well plate reader at 570 nm against a blank reading prepared from gels without cells. Five samples were seeded per experiment. Two independent experiments were performed resulting in a sample size of 10. For statistical analysis, the non parametric Kruskal-Wallis test was performed to determine significant differences in cell viability between the control (**MDP1**) and modified peptides (**MDP2**, **4** and **5**).

To determine whether the presence of the enzyme-cleavable site would encourage cell migration into the hydrogels, SHED were incubated with membrane-permeable fluorescein diacetate (Cell Tracker Green CMFDA, Invitrogen, Carlsbad CA) for 30 minutes, then washed with PBS, detached and seeded on top of hydrogels of **MDP1** or the cleavage-peptide **MDP4**. Cells were seeded on top of pre-formed gels at a density of 5×10^4 cells, which were allowed to adhere for 24 hours. Samples were fixed in 2% paraformaldehyde and embedded for cryosectioning after 1 and 5 days in culture. Sections of 10 μm thickness were prepared on a cryostat microtome, mounted on slides and stored at -20°C. Before use, cells were permeabilized with 5% Triton × in PBS, and cell nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole). Cells and hydrogels were visualized using a Zeiss LSM 510 Meta confocal microscope with an attached PMT.

Results and Discussion

Peptide Design

Previously we have shown that $K_2(SL)_6K_2$ (**MDP1**) has excellent self-assembly and gelation characteristics making good nanofibers with a diameter of approximately 6nm and gels which undergo sheer thinning and recovery.[15] This peptide design was modified to

create four different variants with MMP-2 specific cleavage motif, an RGDS adhesion sequence and either one or two lysine residues in the flanking regions (see table 1).

Specificity of enzymatic cleavage by endopeptidases arises from the amino acid sequence. Occurrence of specific residues 5 positions N-terminal and 3 positions C-terminal from the scissile bond (P5′ – P3) has been demonstrated to affect recognition and increase the specificity of an enzyme for its substrate.[17] The consensus MMP-motif LRG (leucine-arginine-glycine) was chosen based on cleavage site motifs described previously.[17] The sequence SLSLRG was conveniently incorporated into the central block with modification of only two amino acids of **MDP1**. In order to compensate for what was expected to be an unfavorable influence on self-assembly and gel formation of the arginine residue on the hydrophilic face of the peptide and glycine on the hydrophobic face, the amphiphlic B block was elongated by one (SL) repeat.

After assessment of solubility, gelation properties and confirmation of enzymatic cleavage of these peptides, **MDP4** was chosen for further cell culture studies. Along with **MDP1**, these peptides were synthesized with the cell adhesion motif RGD, resulting in **MDP**s **2** and **5**. An N-terminal glycine was added as a spacer, and a C-terminal serine was added to increase the specificity of this motif. [20] The expected fragments after enzymatic cleavage were also synthesized (**MDP4a** and **4b**) to assess their structural characteristics, particularly the possibility that they could still assemble and form β-sheet fibers or gels.

Rheological Properties

The storage modulus G′ and the loss modulus G″ were determined for all MDPs using oscillatory stress sweep analysis (Figure 1). G′ for **MDP**s **2** and **3** were found to be significantly lower than **MDP1**. In the case of **MDP3** (G′ = 43 Pa) the handling properties were poor enough that it would be impractical to use as we have found that hydrogels with storage modulus below 80 Pa are too weak to survive even gentle handling such as washing and exchanging of buffers. To resolve this problem, the flanking region of lysine repeats were shortened in **MDP4** and **5**. The reduced electrostatic repulsion from these amino acids was expected to result in an improved G′ and this was in fact observed to be the case as **MDP4** and **5** have storage moduli of just over 175 Pa at 1% by weight. This illustrates the modular nature in which this class of peptides can be designed and modified. The data presented here refer to peptide gels of 1 % by weight, but substantially stronger gels can easily be produced by increasing the peptide concentration.

Degradation of Peptides with Enzyme-cleavable Site

Figure 2 shows the weight loss of **MDP4** compared to the parent **MDP1**. For both, incubation with PBS resulted in a weight loss of 5 – 10% over a 2-week period. This amount of loss is probably due to small amounts of peptide which are washed off due to daily changes of the supernatant. Incubation with collagenase IV resulted in complete digestion of **MDP4** after 14 days, whereas the control peptide was reduced by only 30% of its original weight. Whereas a lower storage modulus of **MDP4** compared to **MDP1** may be a contributing factor to its more rapid weight loss, we beleive the major difference is due to its enzyme cleavage-site. This is based on observations of a similar peptide (data not shown) with a cleavage-site and a storage modulus was well below that of **MDP4** (68 Pa vs 185 Pa), yet its degradation rate was nearly identical. This peptide was later eliminated from this study due to its low mechanical stability.

Trypsin can also be used to digest the peptide, although at a slower rate than collagenase IV, where 75% of the gel is degraded after 2 weeks. This rather unspecific protease present in the digestive tract cleaves C-terminally of lysine or arginine and is commonly used to detach

adherent cells from tissue culture plates. The effect of hydrogel degradation by trypsin might be interesting for cell culture applications, where entrapped cells could be separated from the gel. Mechanical disruption and dilution of hydrogels in trypsin solution accelerates the digestion process dramatically and enables harvesting live cells for further experiments.

Upon mixing a 2 weight % solution of **MDP**s **1**, **4** or **5** with MMP-2 in PBS at a ratio of 1:1, hydrogels form, but **MDP 4** and **5**, which contain the enzyme cleavage site, are degraded after 48 hrs of incubation at 37°C. This rapid degradation is in contrast to what is observed if the enzyme is merely placed on top of a gel and is likely due to the much higher area of contact obtained by the mixing process. **MDP1** is not affected. MALDI-TOF mass spectroscopy was performed on the peptides mixtures (Figure 3). **MDP1** shows the expected full length peptide peak before as well as after incubation with MMP-2. In contrast, the cleavage peptide shows one peak corresponding to the mass of the peptide molecule before incubation, but many fragments afterwards. For **MDP4**, the peak with the highest intensity could be identified as the N-terminal fragment, KSLSLS, the C-terminal fragment, LRGSLSLSLK is also observed as a small peak. Several additional species were present, many of which corresponded to smaller peptide fragments present in the mixture. The diversity of peptides observed may be attributed to the fact that after cleavage into the two main fragments, the smaller peptide molecules are susceptible to further degradation. Some of the peaks could not be identified as cleavage fragments, and might be due to non-specific digestion of the enzyme itself.

Degradation of **MDP4** nanofibers was visualized by cryo-TEM as illustrated in Figure 4. Whereas **MDP1** remains intact after incubation with MMP-2, **MDP4** transforms from a dense fibrous network to amorphous aggregated remnants, suggesting cleavage and subsequent disintegration of the hydrogel. Neither **MDP4a** nor **4b** which are analogous to the expected N- and C-terminal cleavage fragments formed gels. This indicates that the truncated peptides after degradation of the starting material are no longer able to assemble and form insoluble aggregates, which is a critical point for any *in vivo* applications. Additionally, neither of these fragments displayed a CD spectra characteristic of a β-sheet, but instead display a simple random coil signature (see supporting information).

Two different procedures were used to characterize the profile of enzymatic digestion. Addition of collagenase IV on top of the hydrogels resulted in a degradation profile showing continuous mass loss, indicating surface erosion of the material. For bulk degradation, an initial plateau phase would be expected, followed by a rapid drop of the curve. Surface degradation is the preferred mechanism for many biological applications as it allows migrating cells to locally degrade the matrix as they migrate while overall mechanical integrity of the scaffold is maintained until sufficient amounts of ECM have been produced to replace the synthetic material.

Mixture of hydrogels with MMP-2 results in more rapid disruption of the fibrous network, as can be expected due to the increased surface area for the enzyme to attack, and higher specificity of MMP-2. Crude collagenase contains a varying mixture of enzymes and digests a wider range of substrates. The results after incubation with collagenase IV therefore show proteolytic susceptibility of variant MDP, whereas the digestion with MMP-2 confirms specificity of this enzyme towards the substrate we created. This series of experiments confirms that specific digestion of MDPs occurs when required amino acid residues are present in certain positions relative to the scissile bond: a hydrophobic amino acid at P1′ followed by a basic amino acid and a small residue.

The degradation rate of the peptide hydrogels in a biological setting will depend on many variables, including cell seeding density, susceptibility of the cleavage site, amount of the

targeted matrix metalloproteinase synthesized by the cells, and presence of growth factors. In order to adjust cell-mediated matrix degradation and cellular ECM production to ensure sufficient and necessary support of cells by the synthetic matrix, both parameters need to be monitored under a variety of conditions. For example, faster degradation could be induced by higher cell numbers, slower degradation could be achieved by incorporation of peptides with cleavage sites for other MMPs, which are expressed in smaller quantities in the targeted cells or tissues.

Following these experiments, **MDP4**, which has the same number of amino acids as the parent peptide **MDP1**, is susceptible to enzymatic cleavage and able to form stable hydrogels at physiological pH, was chosen for further analysis as well as modification for cell culture studies.

Influence of the cleavage site on cell proliferation and spreading

In order to investigate the compatibility of the parent and variant peptides with living cells, both **MDP1** and **MDP4** were modified and synthesized with the cell adhesion motif RGD at the C-terminus. Viability was enhanced in **MDP2**, **4** and **5** (which contain either the adhesion motif, the enzymatic cleavage site or both, respectively) compared to **MDP1**. This difference was significant for all time points (day 3, 7 and 14). The highest metabolic activity and viability was observed for cells encapsulated in **MDP5**, suggesting that the presence of an enzyme-cleavable site along with the cell adhesion creates the most favorable environment for cells.

The benefit of both adhesion sequence and cleavage sequence was more strongly demonstrated visually after encapsulation of green fluorescent cells into the hydrogel. Whereas spreading could barely be observed in **MDP1** hydrogels, cell morphology changed slightly in **MDP4**, visibly in **MDP2**, but the biggest change could be observed in **MDP5**, where the combination of cleavage site and cell adhesion motif seem to have a synergistic effect on cell spreading (Figure 6). This is in accordance with previous work, which suggested that initial cell spreading is dependent on proteolytic susceptibility[21] of the matrix. The presence of an MMP-2 cleavage site also dramatically influenced cell migration into MDP hydrogels. When SHED were seeded on top of the gels created from **MDP1** vs. **MDP4**, cells migrated into the **MDP4** hydrogel to a much greater extent (Figure 7).

In conclusion, incorporation of an MMP-2 specific cleavage motif in β-sheet forming peptides resulted in enzyme-mediated digestion and collapse of the hydrogel due to disruption of the nanofibrous network. Furthermore, we demonstrate an immediate effect of the peptide design on cellular behavior, where incorporation of the cleavage site markedly enhanced cell viability, spreading and migration. This specific interaction of cells with the synthetic matrix surrounding them is another step towards the development of ECM-mimics. The versatility of MDPs leaves a variety of possibilities for further modifications and design of custom-made scaffolds for regenerative medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

SHED were kindly provided by Dr. Songtao Shi at USC. This work was funded in part by the Robert A. Welch Foundation (C1557), NSF CAREER Award (DMR-0645474), the Camille Dreyfus Teacher Scholar Awards Program and the 2009 IADR / GlaxoSmithKline Innovation in Oral Care Award. KRR was supported on NIH/NIDCR (T32 DE018380).

References

1. Nicodemus GD, Bryant SJ. Tissue Eng 2008;14:149.
2. Stamenkovic I. J Pathol 2003;200:448. [PubMed: 12845612]
3. Aimes RT, Quigley JP. J Biol Chem 1995;270:5872. [PubMed: 7890717]
4. Shu XZ, Ahmad S, Liu Y, Prestwich GD. J Biomed Mater Res A 2006;79:902. [PubMed: 16941590]
5. Almany L, Seliktar D. Biomaterials 2005;26:2467. [PubMed: 15585249]
6. Hartgerink JD, Beniash E, Stupp IS. Proc Nat Acad Sci 2002;99:5133. [PubMed: 11929981]
7. Holmes TC, de Lacalle S, Su X, Liu G, Rich A, Zhang S. Proc Nat Acad Sci 2000;97:6728. [PubMed: 10841570]
8. McHale MK, Setton LA, Chilkoti A. Tissue Eng 2005;11:1768. [PubMed: 16411822]
9. Seliktar D, Zisch AH, Lutolf MP, Wrana JL, Hubbell JA. J Biomed Mater Res A 2004;68:704. [PubMed: 14986325]
10. Lee SH, Moon JJ, Miller JS, West JL. Biomaterials 2007;28:3163. [PubMed: 17395258]
11. Jun HW, Yuwono V, Paramonov SE, Hartgerink. J Adv Mater 2005;17:2612.
12. Chau Y, Luo Y, Cheung AC, Nagai Y, Zhang S, Kobler JB, Zeitels SM, Langer R. Biomaterials 2008;29:1713. [PubMed: 18192002]
13. Pakstis LM, Ozbas B, Hales KD, Nowak AP, Deming TJ, Pochan D. Biomacromolecules 2004;5:312–8. [PubMed: 15002989]
14. Dong H, Paramonov SE, Aulisa L, Bakota EL, Hartgerink JD. J Am Chem Soc 2007;129:12468. [PubMed: 17894489]
15. Aulisa L, Dong H, Hartgerink JD. Biomacromolecules 2009;10:2694–2698. [PubMed: 19705838]
16. Segers VFM, Lee RT. Drug Discovery 2007;12:561.
17. Turk BE, Huang LL, Piro ET, Cantley LC. Nat Biotechnol 2001;19:661–7. [PubMed: 11433279]
18. Yamada KM. J Biol Chem 1991;266:12809–12. [PubMed: 2071570]
19. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. Proc Nat Acad Sci 2003;100:5808.
20. Hirano Y, Okuno M, Hayashi T, Goto K, Nakajima A. J Biomater Sci Polymer Ed 1993;4:235.
21. Dikovsky D, Bianco-Peled H, Seliktar D. Biophys J 2008;94:2914. [PubMed: 18178662]

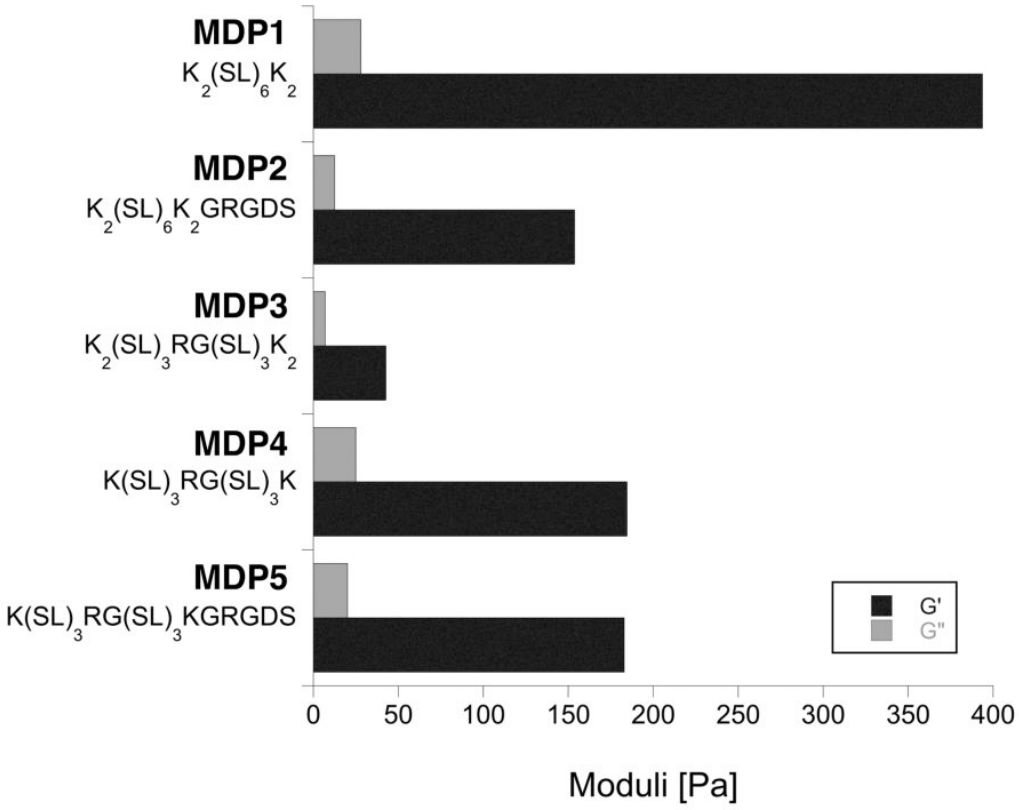

Figure 1.
Storage (black) and loss moduli (grey) of MDPs 1-5 containing variants with and without cleavage sequence, cell adhesion motif and variable length flanking region.

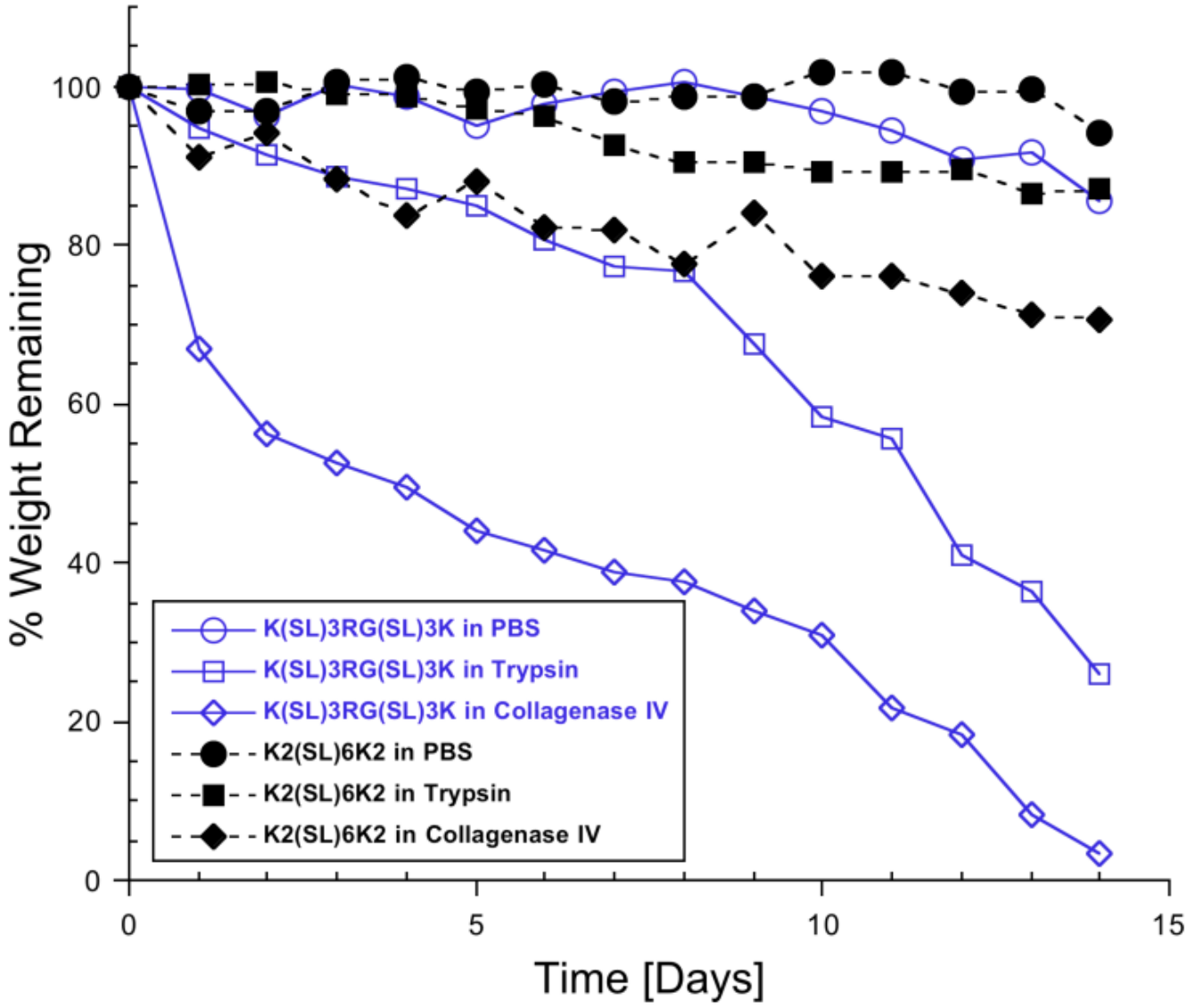

Figure 2.

Hydrogels from **MDP4** (blue, open) and **MDP1** (black, filled) as a control were incubated with trypsin (squares) or collagenase IV (diamonds) and compared to a negative control in PBS. The weight of the hydrogels were determined each day. Data points show averages of three samples. Weight loss is depicted as a percentage where the weight at the beginning of the experiment is 100%.

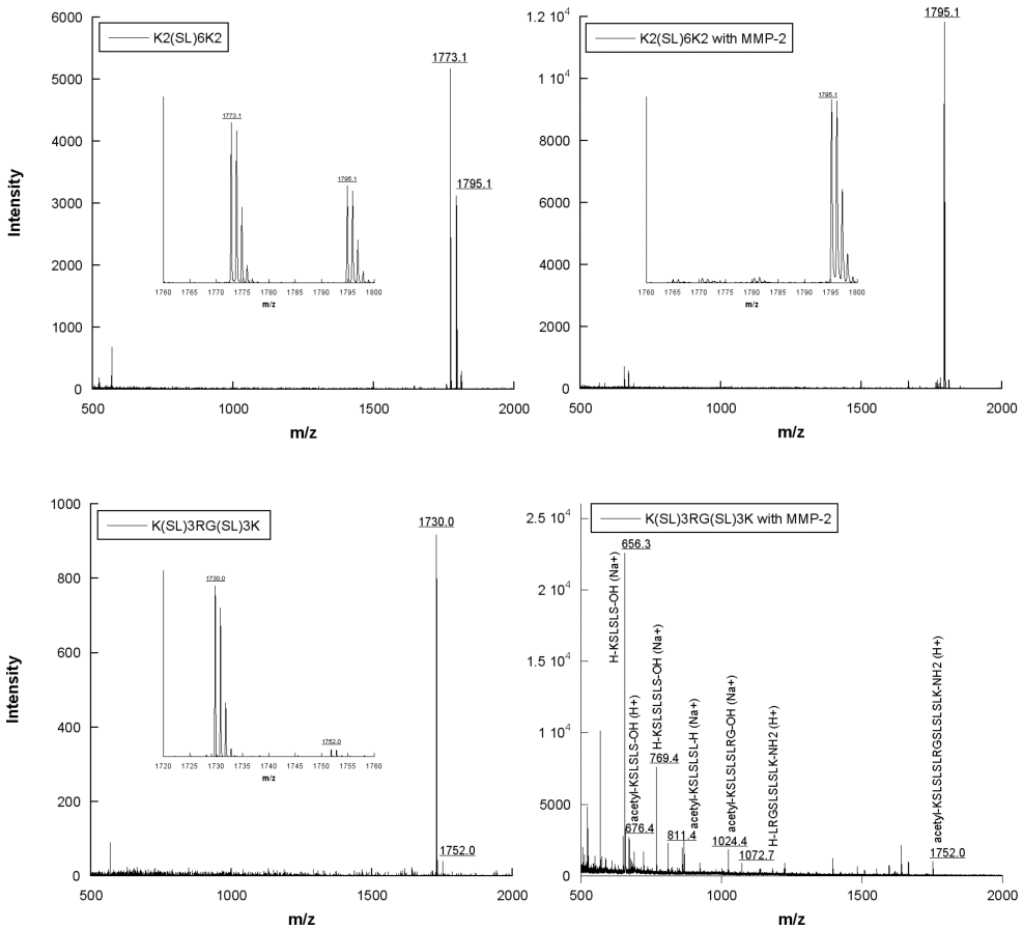

Figure 3.

MALDI-TOF mass spectrometry of MDP1 and 4 before and 48 hours after incubation with MMP-2. Whereas the control peptide shows a single peptide before and after digestion, the cleavage peptide is degraded and multiple fragments can be observed. The expected fragments after cleavage KSLSLS and LRGSLSLSLK are both present. Additional fragments could be identified as alternative cleavage products, whereas some of the peaks remained unidentified.

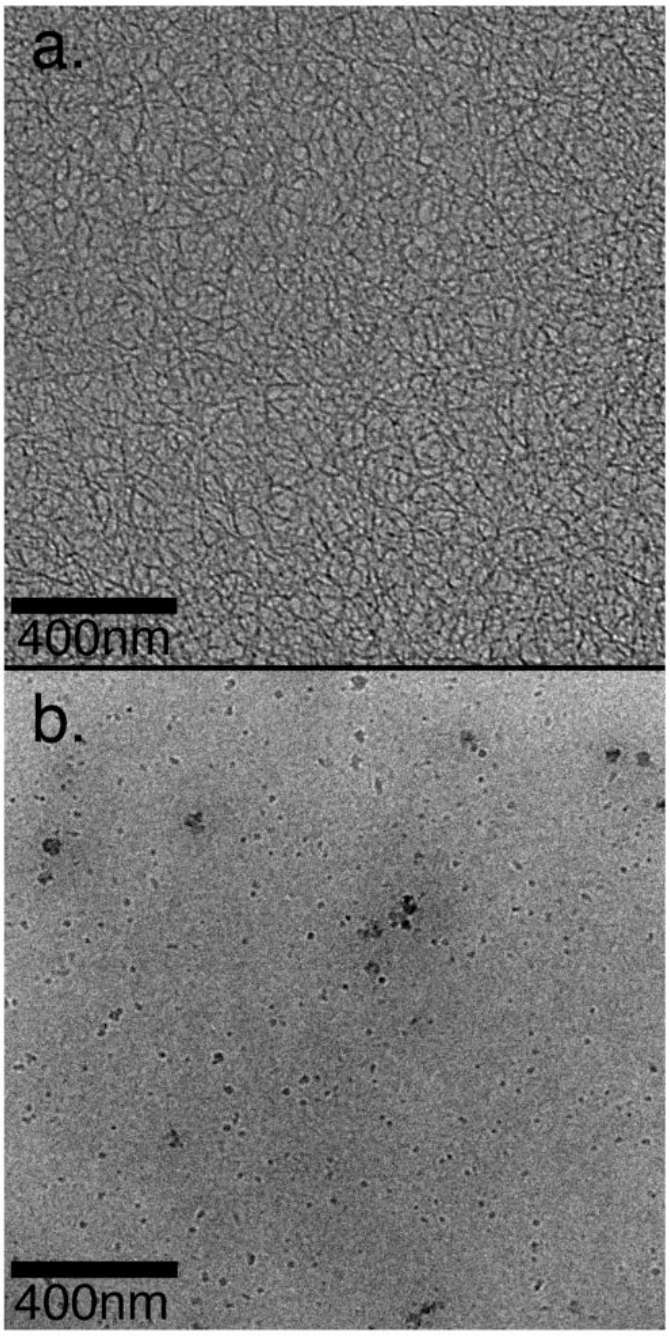

Figure 4.
CryoTEM images before (a) and after (b) incubation with MMP-2, the nanofibers disintegrate leaving small aggregated structures which do not crosslink.

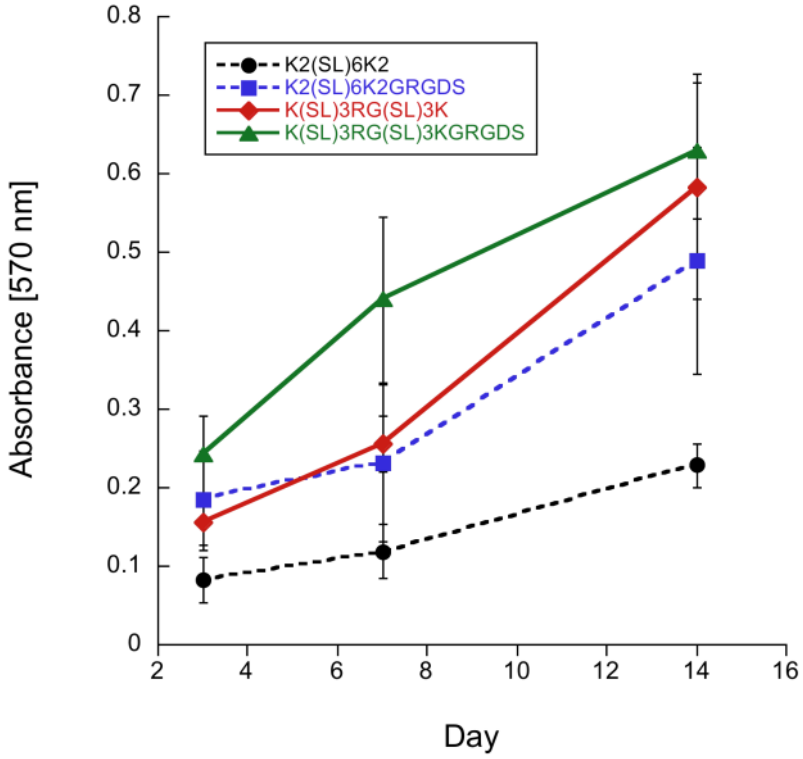

Figure 5.

Cell viability of in MDP1, 2, 4 and 5 as determined by MTT assay after 3, 7 and 14 days in culture. Symbols and bars represent mean values and standard deviations (n=10). Viability is significantly lower in the unmodified peptide, presence of the cell adhesion motif and the cleavage site increase cellular activity, the combination of both in MDP5 results in highest viability and indicates that this is the most favorable environment for cell proliferation among these four peptide hydrogels.

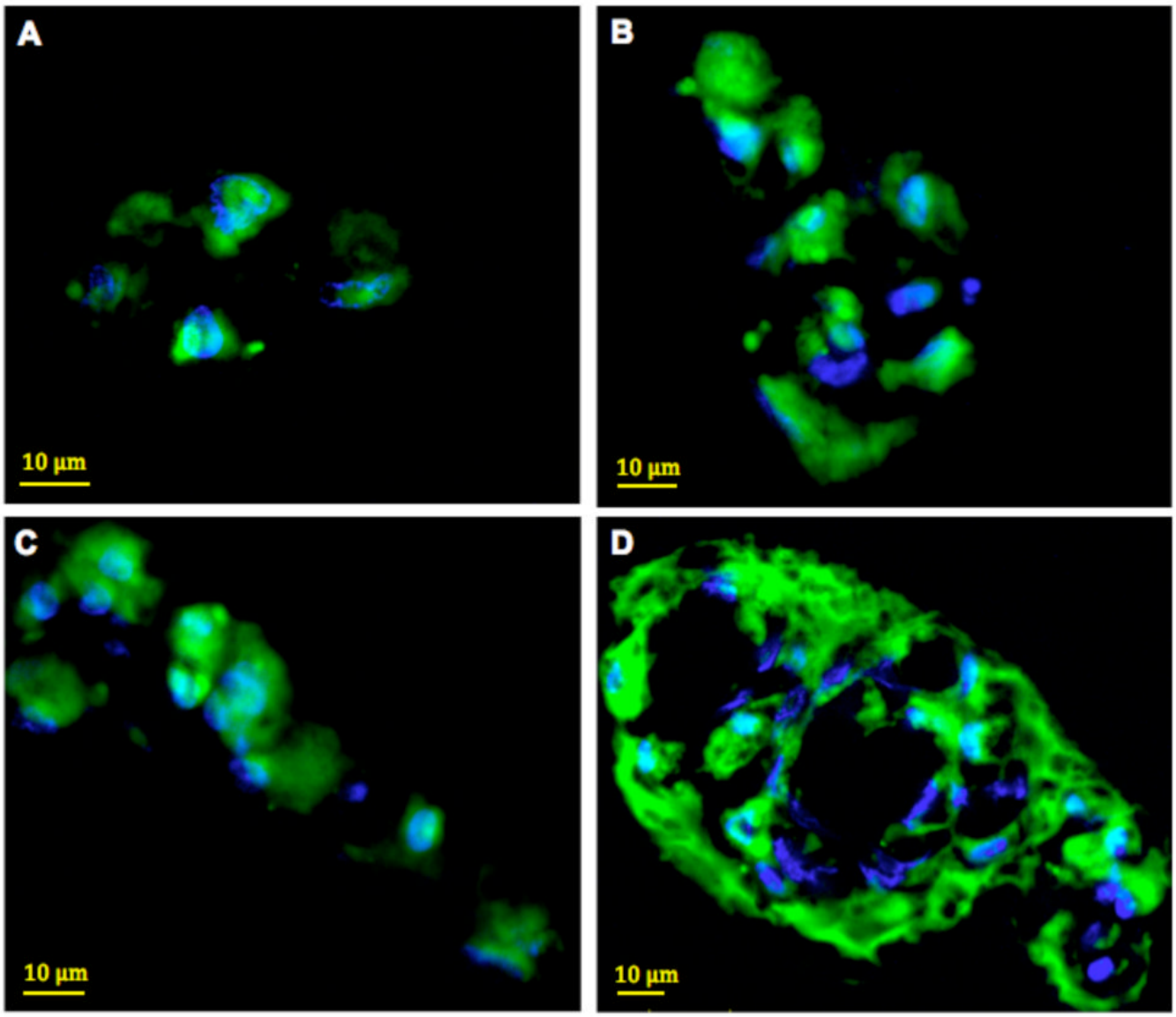

Figure 6.
Fluorescently labeled cells visualized by confocal microscopy (magnification 63×) barely spread in the parent peptide MDP1 (A), but they show enlarged cell bodies when either the cell attachment motif (B) or the cleavage site is present (C). Combination of both bioactive peptide motifs results in enhanced cell spreading (D).

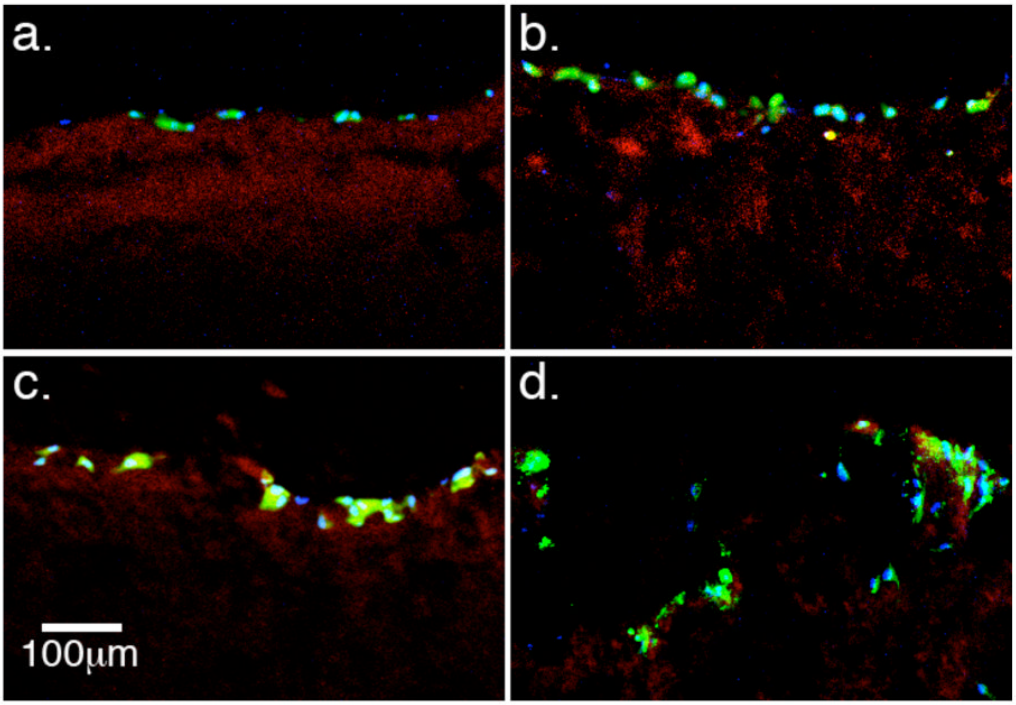

Figure 7.
Green-fluorescent cells were seeded on top of **MDP2** without cleavage site (A, B) and **MDP5** where the cleavage motif is present (C, D). Images show cells after 1 day (A, C) and after 5 days (B, D) in culture. Whereas cells remain as a monolayer on top of **MDP2** hydrogels, they migrate into **MDP5**.

Table 1

Names and sequences of peptides studied.

MDP #	Sequence
1	KK-SLSLSLSLSLSL—KK
2	KK-SLSLSLSLSLSL—KKGRGDS
3	KK-SLSLSLRGSLSLSL-KK
4	K-SLSLSLRGSLSLSL-K
4a	K-SLSLS
4b	LRGSLSLSL-K
5	K-SLSLSLRGSLSLSL-KGRGDS