Molecularly engineered self-assembling membranes for cell-mediated degradation.

Ferreira, DS; Lin, YA; Cui, H; Hubbell, JA; Reis, RL; Azevedo, HS

Advanced Healthcare Materials

Molecularly Engineered Self-assembling Membranes for Cell-mediated Degradation

--Manuscript Draft--

Manuscript Number:	adhm.201400586R1
Full Title:	Molecularly Engineered Self-assembling Membranes for Cell-mediated Degradation
Article Type:	Full Paper
Section/Category:	
Keywords:	peptide amphiphiles; Hyaluronan; self-assembling membranes; matrix metalloproteinase-1; enzyme-responsive materials
Corresponding Author:	Helena S Azevedo, PhD Queen Mary University of London London, UNITED KINGDOM
Additional Information:	
Question	**Response**
Please submit a plain text version of your cover letter here. **If you are submitting a revision of your manuscript, please do not overwrite your original cover letter. There is an opportunity for you to provide your responses to the reviewers later; please do not add them here**.	Dear Dr. Stimson, We are submitting for your consideration the paper entitled Molecularly Engineered Self-assembling Membranes for Cell-Mediated Degradation as full paper for publication in Advanced Healthcare Materials. This paper describes the development of self-assembling membranes that are responsive to cellular activities, in particular to hydrolytic degradation by specific enzymes. This feature is achieved by combining the matrix polymer hyaluronan and a rationally designed peptide amphiphile containing a proteolytic domain (sensitive to matrix metalloproteinase-1, MMP-1). The membranes were shown to be responsive to enzymes activities, in particular to hyaluronidase and MMP-1, and were completely degraded after 7 days in presence of these enzymes. Fibroblasts were used to assess cell-mediated degradation of the membranes and were shown to produce higher amounts of MMP-1 when cultured on MMP-sensitive membranes than when cultured on control membranes. These findings suggest that functionalized membranes stimulate protease secretion, leading to cell-mediated degradation. We believe this work is novel in various ways. First, the functionalization of self-assembling peptides with a MMP-1 sensitive sequence for enzyme-mediated degradation is reported for the first time in this study. Other studies have been focused in the incorporation of MMP-2 cleavable peptide, while the MMP-1 sensitive substrate has been mainly used in the functionalization of 3D polymer gels. Second, the presentation of enzymatic cleavable sequences on self-assembling membranes has never been reported. Moreover, we have designed an enzyme-responsive membrane, enabling the complete degradation of its components by cell-secreted enzymes. This is a major achievement on biomimetic design of artificial matrices for tissue engineering applications. Taken together, we believe that the publication of this study is timely and highly relevant to the specialized readership of Advanced Healthcare Materials.
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Queen Mary University of London
Corresponding Author's Secondary Institution:	

First Author:	Daniela S. Ferreira
First Author Secondary Information:	
Order of Authors:	Daniela S. Ferreira
	Yi-An Lin
	Honggang Cui, PhD
	Jeffrey A. Hubbell, PhD
	Rui L. Reis, PhD
	Helena S Azevedo, PhD
Order of Authors Secondary Information:	
Abstract:	Here we report the use of peptide engineering to develop self-assembling membranes that are responsive to hydrolytic degradation by specific enzymes. The membranes are obtained by combining hyaluronan (HA) and a rationally designed peptide amphiphile (PA) containing a proteolytic domain (GPQGIWGQ) sensitive to matrix metalloproteinase-1 (MMP-1). We found that the insertion of an octapeptide does not disturb PA self-assembly into nanofibers. In the presence of high molecular weight HA, this PA forms self-assembling membranes exhibiting a nanofibrillar morphology. Membrane enzymatic degradation was investigated in vitro in presence of hyaluronidase and MMP-1. Our studies reveal that membranes containing the MMP-1 substrate exhibit enhanced enzymatic degradation, compared with control membranes, being completely degraded after 7 days. Cell culture studies on the developed membranes, using human dermal fibroblasts (hDFbs), show that cell viability and proliferation is minimally affected by the enzymatically cleavable functionality. However, the presence of MMP-1 cleavable sequence does stimulate the secretion of MMP-1 by hDFbs and interfere with matrix deposition, particularly the deposition of collagen. By showing cell-responsiveness to biochemical signals presented on self-assembling membranes, this study highlights the ability of modulating certain cellular activities through matrix engineering.

Molecularly Engineered Self-assembling Membranes for Cell-Mediated Degradation

Daniela S. Ferreira[1,2,3,6], Yi-An Lin[4,5], Honggang Cui[4,5], Jeffrey A. Hubbell[6,7], Rui L. Reis[1,2], Helena S. Azevedo[1,2,3*]

[1] *3B's Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal*

[2] *ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal*

[3] *School of Engineering and Materials Science, Queen Mary, University of London, Mile End Road, London, E1 4NS, UK.*

[4] *Department of Chemical and Biomolecular Engineering*

[5] *Institute for NanoBioTechnology, The Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, United States*

[6] *Institute for Bioengineering, School of Basic Science, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne CH-1015, Switzerland*

[7] *Institute for Molecular Engineering, University of Chicago, Chicago, Illinois 606037, United States*

*corresponding author: h.azevedo@qmul.ac.uk

Tel: +44(0)20 7882 5282, Fax: +44(0)20 7882 3390

Abstract

Here we report the use of peptide engineering to develop self-assembling membranes that are responsive to cellular activities, in particular to hydrolytic degradation by specific enzymes. The membranes are obtained by combining the matrix polymer hyaluronan (HA) and a rationally designed peptide amphiphile (PA) containing a proteolytic domain (GPQGIWGQ octapeptide) sensitive to matrix metalloproteinase-1 (MMP-1). The self-assembly behavior of the designed MMP-1 sensitive PA was studied by circular dichroism (CD) spectroscopy, transmission electron microscopy (TEM) and ability to form membranes with HA. We found that the insertion of an

octapeptide in a typical PA structure does not disturb its self-assembly process into fibrillar nanostructures. CD shows the formation of β-sheet secondary structures in a broad pH range and TEM reveals the presence of filamentous nanostructures. In the presence of high molecular weight HA, this PA forms self-assembling membranes exhibiting a nanofibrillar morphology. Membrane enzymatic degradation was then investigated *in vitro* in presence of exogenous enzymes (hyaluronidase and MMP-1). Our studies reveal that membranes containing the MMP-1 substrate exhibit enhanced enzymatic degradation, compared with control membranes (absence of MMP-1 cleavable peptide or containing a MMP-1 insensitive sequence), being completely degraded after 7 days. Cell culture studies on the developed membranes, using human dermal fibroblasts (hDFbs), show that cell viability and proliferation is minimally affected by the enzymatically cleavable functionality present on the membrane. However, the presence of MMP-1 cleavable sequence does stimulate the secretion of MMP-1 by hDFbs and interfere with matrix deposition, particularly the deposition of collagen. By showing cell-responsiveness to biochemical signals presented on self-assembling membranes, this study highlights the ability of modulating certain cellular activities through matrix engineering. We believe this concept can be further explored to understand the cellular remodeling process in different tissues and could also be used as a strategy to develop artificial matrices with more biomimetic degradation for tissue engineering applications.

Keywords: peptide amphiphiles; hyaluronan; self-assembling membranes; matrix metalloproteinase-1; degradation; enzyme-responsive materials

1. Introduction

The specific and selective activities of enzymes, together with their ability to be controlled by environmental conditions, such as pH, temperature and ionic strength, have been extensively explored in the materials science community with the aim of producing materials with customized functionalities while offering the possibility to perform catalysis reactions very efficiently in mild conditions. According to a recent review published by Ulijn and co-workers on enzyme responsive materials (ERMs),[1] they define ERMs as materials that change their functionality as a result of the direct action of an enzyme on the material. The ubiquitous distribution and great diversity of enzymes present in the human body has motivated biomedical engineers to design biomaterials responsive to enzyme activities and thus translate the enzymatic modification into a suitable materials response. Because the trigger is provided by the biological environment (e.g. cells), with no need to be added exogenously, ERMs offer additional advantages for biomedical applications when compared with other stimuli-responsive (e.g. light, temperature) materials. Enzymes can either be used to form new covalent bonds (e.g. isopeptide bonds between side groups of amino acids in peptides and proteins by transglutaminase, leading to the cross-linking and strengthening of the material), or the cleavage of pre-existing bonds (e.g. hydrolysis of peptide bonds by proteases, leading to the material degradation or disassembly). In the context of 3D biomaterials for cell culture, enzymes have been mainly used to form polymer hydrogels, by incorporating enzyme sensitive cross-links and thus induce gelation *in vivo*, or, conversely, to mimic the degradation process during tissue remodeling and favor cell migration and invasion through enzyme sensitive polymer hydrogels. Both approaches were pioneered by the Hubbell's lab to develop polyethylene glycol (PEG)-based hydrogels with different functionalities.[2, 3] These concepts were then applied to other natural[4, 5] and synthetic[6] polymer hydrogels. Similarly, enzyme responsive supramolecular hydrogels were developed, including the formation of gels that are obtained by enzyme-directed self-assembly[7] (an enzyme is used to catalyze the synthesis of

self-assembling molecules or the removal of a block to trigger the self-assembly), and the degradation[8, 9, 10] of supramolecular gels by enzyme mediated disassembly (enzyme converts the self-assembling blocks into non-self-assembling molecules or by degrading the building block directly).[1] Selective enzymatic degradation was also shown to be advantageous in modulating the properties of self-assembled nanostructures, such as nanostructure transitions. For example, Webber and co-workers designed a peptide amphiphile (PA) containing a sequence that is a substrate of a protein kinase A (PKA).[11] Upon treatment with PKA, the PA molecules became phosphorylated causing the disassembly of the original cylindrical structures. Subsequent treatment with alkaline phosphatase enzyme, which cleaves the phosphate groups, resulted in PA reassembly. Seitsonen and co-workers used the model protease α-chymotrypsin to control the morphologies of peptide self-assemblies (nanotubes/helical ribbons, spherical micelles).[12] In a different approach, Lin and co-workers used a specific proteinase to degrade a cross-linker that stabilizes supramolecular peptide filaments.[13]

Normally during granulation tissue formation, the remodeling of the natural extracellular matrix (ECM) occurs simultaneously with cell invasion. The macromolecular components of the ECM are degraded by cell-secreted proteases, mainly by matrix metalloproteinases (MMPs). The MMPs are a family of protease enzymes that are able to cleave structural components of the ECM, such as collagen types I and IV and fibrin, playing an important role in morphogenesis, wound healing and tissue remodeling.[14] In general, MMPs are not continuously expressed in the skin, but are induced temporarily in response to exogenous signals or pathogenic conditions, such as wound healing.[15] In later stages of dermal wound healing, fibroblasts are the main cell population that produce native ECM[16] and secrete active MMPs (MMP-1, -2, -9 and -13).[15, 17] The action of MMP-1 is essential in the remodeling stage during wound healing.[18] This enzyme is an antagonist to the ECM synthesis and therefore mediates the balance between tissue synthesis and degradation. Several attempts in biomaterial design have been made to mimic this feature of natural ECMs by

including protease-sensitive peptides that are cleaved in the presence of cell-secreted enzymes, thus providing further control over cellular responses.[2, 19] The inclusion of MMP-sensitive sequences into PEG gels has been explored by Hubbell's lab to promote cell migration within hydrogels.[2, 20, 21] When the gels come into contact with cells *in vitro* or *in vivo*, they were locally degraded as the cells respond to cues presented by the gels. The inclusion of MMP cleavable sequences into self-assembling peptides has been explored for different biomedical applications, including enzyme responsive hydrogels for controlled matrix degradation and cell migration in tissue engineering,[8, 9, 22] enzyme-triggered drug delivery systems[23] and sensing systems for detecting cell-secreted MMPs overexpressed in various diseases.[24]

Hyaluronan (HA), a major component of the ECM, has attracted considerable interest in a wide range of biomaterials applications.[25] Its unique physicochemical properties[26] (high molecular weight and density of negative charges, inherent biodegradability and biocompatibility), associated with its complex interactions with ECM components and cells,[27] have led us and others to consider this simple polymer as building block for designing biomimetic materials. Moreover, HA has shown to play important roles in wound healing.[28]

As bottom-up approach, self-assembly allows the design of biomaterials with specific functionalities and nanoscale organization similar to natural tissues. The formation of hierarchically ordered membranes by self-assembly between high molecular weight HA and positively charged peptide amphiphiles (PAs) was first reported in the seminal work of Stupp and co-workers.[29] We have then used the same approach to fabricate membranes integrating biochemical signals (RGDS ligand) to enhance the adhesion of fibroblasts in serum-free culture conditions.[30] To further expand the versatility of these self-assembling membranes as temporary matrices for wound healing applications, and recreate some of the aspects of matrix remodeling, an enzymatic cleavable peptide sequence has now been included into the peptide domain. The

inclusion of this sequence allows the creation of membranes that are degraded in response to cell-secreted enzyme activities at the wound site during tissue remodeling.

Therefore, the objective of this study was to develop self-assembling membranes made of HA and PAs containing a cleavable site for MMP-1. Since hyaluronidase and MMP-1 enzymes are released by fibroblasts during wound healing, we further examined the ability of these enzymes to degrade the self-assembling membranes. The biological functionality of the membranes was also investigated by culturing human dermal fibroblasts and their effect on cell behavior, like cell adhesion, proliferation and ECM synthesis, evaluated.

2. Results and discussion

2.1. Design and self-assembly of PAs with enzymatic cleavable sequences

The self-assembly of HA with positively charged peptides containing the RGDS epitope has previously been explored by our group to generate membranes with different densities of peptide ligands and thus control the adhesion of different cell types.[30, 31] To add different functionalities into our membrane design, such as programmed degradation, a similar molecular engineering approach was pursued in this work by including the matrix metalloproteinase-1 (MMP-1) cleavable site into the peptide building block. The peptide self-assembling blocks used in this study are based on the peptide amphiphile design pioneered by Stupp[32] and are composed of a hydrophobic segment coupled to a peptide segment, that includes a β-sheet forming sequence (V_3A_3) and a domain with positively charged amino acids (K_3) to bind the anionic HA (K_3 PA, Figure 1A).[29] A MMP-1-sensitive PA was designed by incorporating the sequence GPQG↓IWGQ[2, 20, 21, 33] (arrow denotes the expected cleavage site by MMP-1) between the β-sheet domain and the lysine residues (MMP_sK_3 PA, MMP sensitive). This peptide is the mutated version of the sequence GPQG↓IAGQ found within the α chain of type I collagen of calf and chick, as well as human.[34] The single substitution of Ala by Trp was shown to enhance enzymatic cleavage.[2, 35] The human α1 type IV collagen sequence, GDQGIAGF, was chosen as a negative control. This sequence has been described to be insensitive to MMP-1 degradation (MMP_iK_3 PA, MMP insensitive).[2, 36] By inserting the GPQG↓IWGQ segment between the HA-binding region and the β-sheet forming segment in the original PA design, instead of adding it to the C-terminus, we aim to promote the breakdown of the peptide at a point that would facilitate the release of the HA-binding motif from the PA nanofibers, hindering binding to HA to remain bound. The lack of interaction between the PA nanofibers and the HA chains would favor the disassembly and dissolution of the membrane components. In addition to MMP-1, hyaluronidase (HAase) has also been implicated in tissue remodeling during wound healing. The presence of HAase will further contribute for the

membrane disassembly and degradation by hydrolyzing HA into smaller fragments.[30] We hypothesize that the incorporation of enzymatic cleavable building blocks in the formulation of our membranes, enables the complete degradation of its components by cell-secreted enzymes and this process would resemble the breakdown of native ECM during tissue remodeling.

The ability of K_3-PA to form β-sheet secondary structures at certain pHs and to self-assemble into nanofibers was shown previously.[30] However, significant modifications, like the insertion of a sequence containing eight amino acids, may affect the self-assembly properties of the resulting PAs. Profiling the secondary structure of the GPQGIWGQ-containing PA by CD spectroscopy and examining the existence of β-sheets can provide basic information to understand their assembling properties (Figure 1B). The CD spectra of β-sheet structures are typically characterized by a minimum at 216 nm and a maximum at 195 nm, while random coils present a maximum at 218 nm and a minimum at 198 nm.[37] The CD spectra obtained for K_3PA at neutral and basic pH were consistent with the known fact that this peptide has a dominant β-sheet content.[30, 38] The inclusion of the sequences GPGIWGQ and GDQGIAGF between the β-sheet domain and the positively charged lysine residues seems to reinforce the β-sheet behavior of the peptides (Figure 1B). A typical β-sheet signature was observed for both peptides in all the studied pH conditions, with a minimum in the 210-220 nm range, a crossover from positive to negative above 190 nm, and a positive ellipticity around 200 nm (Figure 1B). With exception of proline, all the inserted amino acids are either neutral or β-sheet promoters,[39] thus contributing to the formation of β-sheet structures, independently of the solution pH.

PAs, including these designed in this study, typically self-assemble into high-aspect-ratio filamentous nanostructures. TEM analysis (Figure 1C) confirmed the self-assembly of all PAs into nanofiber structures with diameters of between 12 and 16 nm (Table S1) and lengths of from a few hundred nanometers to several micrometers. Interestingly, MMP_sK_3 PA formed shorter nanofibers in comparison with K_3 and MMP_iK_3 PAs. This might be due to the placement of a proline

residue next to the β-sheet forming peptide region. The cyclic structure of proline's side chain confers exceptional conformation rigidity. Its unique kink formation may add some steric effect on the molecular packing of the adjacent residues, thus limiting the growth of the nanofiber.

2.2. Membrane self-assembly

Recent advances in biomaterials science have enabled the possibility to develop artificial matrices that recapitulate key features of the natural ECM critical for the process of tissue regeneration.[40] Based on our previous studies,[30] we hypothesize that self-assembling membranes with diverse functionalities can be obtained by self-assembly between HA and positively charged PAs through the incorporation of specific sequences recognized by cell receptors or specific enzymes (Figure 1A). Although the insertion of a sequence of eight amino acids into the PA structure did not disturb their self-assembly properties (Figure 1B, 1C) the presence of this additional segment could potentially affect the membrane self-assembly. To prepare the membranes (Figure S4), an aqueous solution containing K_3 PA was added on the top of the HA solution, and left to incubate overnight at the temperature of 37 °C. Similarly, membranes containing the MMP-sensitive (Figure S5) and -insensitive sequences were prepared using MMP_sK_3 PA and MMP_iK_3 PA, respectively. SEM images (Figure 3B1) showed that all membranes present a nanofibrillar structure that resulted from the PA self-assembly in presence of the anionic polysaccharide.

2.3. Membrane degradation by exogenous enzymes

Various studies[4, 21] have showed that non-degradable hydrogels limit cellular infiltration. We have demonstrated that self-assembling membranes containing HA and K_3 PA were susceptible to enzymatic degradation by HAase.[30] The incorporation of MMP-sensitive sequences in our membrane design is expected to promote their degradation by proteolytic enzymes and this would lead to enhanced cellular invasion (Figure 2). Thus, when implanted *in vivo* both

components of the membrane would be susceptible to degradation by enzymes expressed by the surrounding cells (e.g. HAase, MMPs) improving cell migration and tissue morphogenesis.

To assess membrane degradability, membranes containing the MMP sensitive sequence (MMP_sK_3-HA), the insensitive sequence (MMP_iK_3-HA) and the control (K_3-HA) were incubated in PBS in the presence of HAase (50 U mL^{-1}), MMP-1 (10 nM) or both enzymes. HA degradation was followed by quantification of a new reducing N-acetyl-D-glucosamine terminus with each cleavage reaction and membranes were analyzed by SEM (Figure 3). Incubation in PBS up to 14 days did not cause degradation of the membranes, as evidenced by the low amount of N-acetylamino sugars in the supernatants (Figure 3A1, A2), and the absence of evident signs of degradation in the SEM micrographs (Figure 3B1). When incubated in the presence of HAase, membrane degradation was significantly enhanced in all the conditions, which is in agreement with what we have observed before.[30] In the presence of MMP-1, only the MMP_sK_3-HA membranes showed signs of degradation, as seen in the SEM micrographs (Figure 3B1). From day 5, there is a slightly increase in the released N-acetylamino sugars (Figure 3A2). Analyzing the effect of MMP-1 activity on the release of N-acetylamino sugars from MMP_sK_3-HA and MMP_iK_3-HA membranes (Figure 3A3), only the MMP_sK_3-HA membranes showed an increase in the amount of N-acetylamino sugars in solution, while in the MMP_iK_3-HA the amount of N-acetylamino sugars was negligible and remained constant along the time. The increase in the amount of N-acetylamino sugars in solution might be due to the release of HA from the membrane, as the peptide being cleaved by MMP-1 (it breaks down the HA-binding region from the main PA sequence) weakens the interaction with HA. The absence of degradation in the K_3-HA and MMP_iK_3-HA membranes in the presence of MMP-1 suggests the specificity of the enzyme for the MMP substrate (GPQG↓IWGQ) and that the control sequence (GDQGIAGF) is not cleaved by the enzymatic actions of MMPs, as reported previously.[2] In the presence of both HAase and MMP-1, the MMP_sK_3-HA membranes were completely degraded after 14 days, demonstrating the additive effect of both enzymes. At this stage,

membranes underwent extensive degradation and were difficult to recover for SEM analysis, thus SEM micrograph shows a sample only for day 7 (Figure 3B1). SEM analysis of the cross-section of MMP_sK_3-HA membranes in the presence of MMP-1, shows that the degradation was more evident on the surface on the peptide side (Figure 3B2).

2.4. Cell culture studies

Effect of membrane functionalization on fibroblast proliferation

During the early stages of wound healing, fibroblast migration and proliferation into the wound matrix depend on cell-matrix interactions.[18, 41] Previous work suggested that PAs bearing different charges could affect cell viability.[42] To investigate whether some of the sequences could affect fibroblast viability and proliferation, cells were cultured either on glass coverslips coated with different PA molecules or on membranes made with HA and the same PAs. Moreover, the effect of the addition of a broad-spectrum MMP inhibitor (GM6001) in cell proliferation was also investigated, as we intend to use this inhibitor in further experiments. DNA quantification results showed that cells were able to proliferate up to 7 days, either on glass coated coverslips (Figure S4) or on the membranes (Figure 4), which indicates that these PA molecules do not affect cell proliferation. Additionally, cell proliferation was greatly decreased in the presence of the MMP-inhibitor (GM6001, 20 μM), which is consistent with what has been observed by other authors that suggest this inhibitor might have a reversible effect on fibroblast proliferation.[21, 43] Contrary to what has been observed when culturing cells in 3D hydrogels,[9, 21] there was no difference on cell proliferation when cells were seeded on MMP_sK_3-HA membranes versus MMP_iK_3-HA.

Effect of membrane functionalization on MMP-1 secretion by fibroblasts

In later stages of dermal wound healing, fibroblasts are the main cell population that produces native ECM.[16] Cells normally secrete and activate proteolytic enzymes in order to invade or

remodel the extracellular microenvironment.[44] In particular, primary human dermal fibroblasts are known to secrete active MMPs.[17] At this stage, for successful wound healing and prevention of fibrotic tissue, a fine balance between ECM synthesis and degradation is required. The action of MMP-1 is essential during the remodeling stage of wound healing.[18] Here, MMP-1 expression was examined at the protein level using ELISA. Fibroblasts cultured on the membranes were shown to secrete MMP-1. The amounts of MMP-1 released by the cells cultured on the MMP_sK_3-HA membranes were higher than those of cells cultured on the corresponding MMP-insensitive derivatives and on the control (K_3-HA) (Figure 5A), suggesting that the presence of MMP-sensitive motifs in the membrane might have an influence in the secretion of MMP-1. When cultured on peptide coated glass coverslips, however, no differences were observed between different PAs on MMP-1 production (Figure S7). In addition, the values for MMP_sK_3-HA membranes were 2-fold higher than on the coated glass. These findings suggest that the mode of signal presentation to cells may have an influence on their response, a hypothesis that remains to be further investigated. Previous studies have shown the influence of matrices presenting bioactive ligands on the secretion of proteases.[2, 4] As expected, small amount of MMP-1 was detected in the supernatant in the presence of the MMP-inhibitor (Figure 5A). Confocal microscopy images showed elongated cells with typical fibroblast morphology (Figure 5B). After 24 h, MMP-1 was expressed by the cells in all the conditions but seems more evident for MMP_sK_3-HA membranes, which corroborates the ELISA results (Figure 5A).

Production of ECM proteins by fibroblasts cultured on the membranes with different functionalities

The native ECM of the dermis mainly consists of collagens, proteoglycans and adhesive glycoproteins.[45] In wound healing, fibroblasts are essential for the production of new native ECM.[46] To investigate the ability of hDFbs to synthesize native ECM, we determined the amount

of collagen and non-collagenous extracellular matrix proteins produced by the cells when cultured on the different membranes (Figure 6). Except for the MMP_sK_3-HA membranes, there was an increase in collagen and non-collagenous proteins production with culture time in all the conditions. The amount of collagen deposited on MMP_sK_3-HA membranes decreased with time (Figure 6A) while the amount of non-collagenous proteins increased, as observed for the other conditions (Figure 6B). The ECM turnover is controlled by the synthetic rate of matrix proteins, including collagenous and non- collagenous proteins, and their enzymatic degradation by MMPs. In particular, MMP-1 is the only protease able to unwind collagen type I and initiate its degradation.[47] Elevated MMP-1 activity is responsible for an increased turnover of the extracellular collagen matrix.[48] Taken together, the data of collagen and MMP-1 production, suggest that the reduced levels of collagen on MMP_sK_3-HA membranes (Figure 6A) could be due to the increased amounts of secreted MMP-1 (Figure 5A) observed for these membranes. As previously observed for the production of MMP-1, no differences were shown between conditions on the production of collagen when cells were cultured on peptide coated glass coverslips (Figure S8A), suggesting again the effect of matrix microarchitecture and signal presentation on cell behavior.

Membrane microstructure after cell culture

SEM analysis of the membranes after culturing hDFbs showed that cells were able to adhere to and invade the membranes (Figure 7). After 14 days of culture, the microstructure of the MMP-sensitive membranes showed a loose network of nanofibers, when compared with the control membranes (K_3 and $MMP_{insensitive}$). Conversely, when adding the MMP inhibitor into the culture medium, the membrane retained its initial morphology. These results suggest that the underlying membrane is responsive to the enzymatic activities produced by the cells. However, the membranes remained intact after 14 days, implying that cell-triggered proteolysis was localized on

the surface of the membranes into the cell periphery. The localization of the enzymatic degradation on the surface mimics proteolysis *in vivo*, in which ECM degradation is confined to the immediate pericellular environment.[44] An adequate correlation between the membrane degradation and production of MMP-1 is not possible at this point, because other MMPs are secreted by fibroblasts (MMP-2, MMP-13). In addition, a balance between active MMPs and their natural inhibitors (tissue inhibitors of metalloproteinases, TIMPs) could also be responsible for the localized degradation behavior in the membranes. Thus, we cannot conclude at the moment which particular molecules are involved in cell proteolysis, but we have an indication that at least MMP-1 is involved.

The balanced regulation of fibroblast proliferation and ECM remodeling is essential for optimal wound healing results. Our *in vitro* data suggest that cells responded to the functionalized membranes, and were able to produce ECM proteins without extensive matrix deposition. The inclusion of a MMP-sensitive sequence into the membranes increased matrix proteolytic degradability, which would lead to enhanced cellular invasion when implanted *in vivo*, and thus could be beneficial for an enhanced wound healing response. The mechanisms underlying these observations remain, however, unclear and are worthy to be further investigated.

3. Conclusions

Using a molecular engineering approach, we designed a peptide amphiphile capable of self-assembling with hyaluronan into membranes and containing a proteolytic domain sensitive to matrix metalloproteinase-1 (MMP-1). By pursuing this design, we were able to modulate the degradation rate of the formed self-assembling membranes, as well as certain cellular responses. The membranes were shown to be responsive to enzymes activities, in particular to hyaluronidase and MMP-1, and were completely degraded after 7 days in presence of exogenous enzymes. Fibroblasts, very active cells in the wound healing process, were used to assess cell-mediated degradation of the membranes and were shown to produce higher amounts of MMP-1 when cultured on MMP-sensitive membranes than when cultured on the MMP-insensitive and on the control (bare membranes without MMP signal). These findings suggest that membranes containing the MMP-1 cleavable sequence stimulate protease secretion, thus leading to cell-mediated degradation process. This hypothesis, however, needs to be further investigated. We expect that by including components sensitive to enzymatic activities in the membrane formulation, their degradation behavior may resemble the process of ECM remodeling, leading to increased cellular infiltration and ultimately more robust healing *in vivo*. Nevertheless, *in vivo* experiments will still be required to verify that the expected remodeling of the ECM actually takes place. These cell-interactive membranes can be further functionalized with other biochemical moieties to study cellular processes *in vitro* and also as temporary matrices in tissue engineering applications.

Acknowledgments

This work was funded by the European Regional Development Fund (ERDF) through the Operational Competitiveness Programme “COMPETE” (FCOMP-01-0124-FEDER-014758) and national funds through the Portuguese Foundation for Science and Technology (FCT) under the

project PTDC/EBB-BIO/114523/2009. We also thank a start-up grant provided by the School of Engineering and Materials Science at QMUL. D. S. Ferreira gratefully acknowledges FCT for the PhD scholarship (SFRH/BD/44977/2008).

4. Experimental Section

Hyaluronan (HA)

Hyaluronan, with an average molar mass of 1.5 MDa (Lifecore Biomedical, Inc, Chaska, USA) was used in this study for self-assembly with the newly designed peptide amphiphiles.

Peptide amphiphiles: design/structure, synthesis and purification

Three different peptide amphiphiles (PAs) were synthesized in this work, all consisting of a peptide segment covalently linked to a 16-carbon alkyl chain: $C_{15}H_{31}CO$-$V_3A_3K_3$ (K_3 PA), $C_{15}H_{31}CO$-$V_3A_3GPQGIWGQK_3$ (MMP_sK_3 PA) and $C_{15}H_{31}CO$-$V_3A_3GDQGIAGFK_3$ (MMP_iK_3 PA) (Figure 1A). The peptides were synthesized on a CS Bio 136XT automated peptide synthesizer (CS Bio, USA) using standard 9-fluorenylmethoxycarbonyl (Fmoc) based solid phase chemistry on a 4-methylbenzhydrylamine (MBHA) rink amide resin. Amino acid couplings were performed using 4 equivalents (4 mmol) of Fmoc protected amino acids (Novabiochem, USA), 4 equivalents of O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU, Carbosynth, UK) and 6 equivalents of N,N-diisopropylethylamine (DIEA, Sigma, USA). Fmoc deprotections were performed with 20% piperidine (Sigma, USA) in dimethylformamide. A palmitic acid ($C_{16}H_{32}O_2$, Calbiochem, USA) tail was manually coupled under the same conditions as the Fmoc-amino acids. Peptide cleavage from the resin, and removal of the protecting groups, was carried out on a mixture of trifluoroacetic acid (TFA, Sigma, USA)/triisopropylsilane (TIS, Alfa Aesar)/water (95/2.5/2.5)) for 3 h at room temperature. The peptide mixture was collected and excess of TFA was removed by rotary evaporation. The resulting viscous peptide solution was triturated with cold diethyl ether and the white precipitate was collected by filtration, washed with cold ether, and allowed to dry under vacuum overnight. The peptide mass was confirmed by electrospray ionization mass spectrometry (ESI-MS, Agilent, USA).

Peptides were then purified on a Waters 2545 Binary Gradient high-performance liquid chromatography (HPLC) system using a preparative reverse-phase C18 column (Atlantis Prep OBD T3 Column, Waters, USA) and a water/acetonitrile (0.1% TFA) gradient. TFA counter-ions were exchanged by sublimation from 0.01 M hydrochloric acid. Finally, the peptides were dialyzed against ultrapure water using 500 MWCO dialysis tubing (Spectrum labs, The Netherlands), lyophilized and stored at -20 °C until further use. Confirmation of mass and purity was done by ESI-MS and HPLC (Supplementary Information, Figure S1-S3).

Peptide amphiphiles characterization

Circular dichroism (CD) spectroscopy

Peptides were dissolved in deionized water to a final concentration of 0.011 mM and the pH was adjusted to 3, 7 and 9 with hydrochloric acid (0.1 M) or ammonium hydroxide (0.1 M). The CD measurements were performed in a PiStar-180 spectrometer from Applied Photophysics (Surrey, UK), under a constant flow of nitrogen (8 $L.min^{-1}$) at a constant pressure value of 0.7 MPa. Far-UV spectra were recorded at 25 °C from 190 to 300 nm in a quartz cuvette with 1 mm path-length. All scans were performed in the steady state with a bandwidth of 1 nm and each represented spectrum is an average of 5 spectra. The molar ellipticity [θ] was calculated using equation (1).

$$[\theta] = \frac{\theta}{c.l} \qquad (1)$$

were θ is the measured ellipticity in mdeg, c is the concentration of the peptide in dmol L^{-1} and l is the light path length of the cuvette in cm.

Transmission electron microscopy (TEM)

Peptides were dissolved in ultrapure water (0.1 wt%) and the solution was aged for 2 days before TEM analysis. Peptide solutions were loaded on carbon-coated copper TEM grid (Electron

Microscopy Sciences, USA). For negative staining, a drop of 2 wt% uranyl acetate (Electron Microscopy Sciences, USA) aqueous solution was placed on the samples. The excess solution was wiped away by a piece of filter paper, and the sample was allowed to dry under ambient conditions. All images were collected with a Tecnai 12 TWIN, equipped with SIS Megaview III camera (FEI, USA).

Preparation of self-assembled PA-HA membranes containing different functionalities

Self-assembled membranes were prepared by combining HA with positively charged PAs at optimized conditions, as previously reported.[30] Briefly, 50 µL of a 1 wt% HA solution was cast on the bottom of the wells of a 96 well plate and then 50 µL of 0.017 M K_3PA solution was added on top of the HA solution. Similarly, membranes containing the MMP sensitive and insensitive sequences were prepared using MMP_sK_3 and MMP_iK_3 PAs at 0.017 M concentration. The membranes were allowed to develop overnight and rinsed with sterile ultrapure water to ensure the removal of unreacted HA and PA.

Enzymatic degradation of PA-HA membranes by exogenous enzymes (MMP-1 and hyaluronidase)

Degradation studies were carried out by incubating the different PA-HA membranes in PBS at 37 °C for 14 days without enzymes (control) or containing 10 nM of MMP-1 (human MMP-1, EC 3.4.24.7, Sigma, USA) and 50 $U.mL^{-1}$ of bovine testicular hyaluronidase (Type IV, EC 3.2.1.35, Sigma, USA) isolated or combined. The enzyme solutions were replaced every 72 h throughout the study and collected supernatants were stored at -20 °C for further analysis. At predetermined time points, the membranes were collected and their morphology examined by SEM, as described below. HA degradation on the membranes was assessed by quantification of N-acetylamino sugars in the collected supernatants using the fluorimetric Morgan–Elson assay method[49] and a

calibration curve of N-acetyl-D-glucosamine (NAG) standards. Three independent experiments were performed for each degradation condition and incubation time.

Scanning electron microscopy (SEM)

The microstructure of the membranes after degradation was analyzed by SEM. The membranes were first fixed in 2% glutaraldehyde/3% sucrose in PBS for 1 h at 4 °C followed by sequential dehydration in graded ethanol concentrations (from 20 to 100%). To remove ethanol, samples were dried in a critical point dryer (Tousimis Autosandri®-815 series A, USA). Prior observation, the samples were coated with a 15 nm thick layer of gold/palladium and imaged using an ultra-high resolution field emission gun scanning electron microscope (Nova™ NanoSEM 200) from FEI (Eindhoven, The Netherlands).

Cell culture studies

Isolation and culture of primary human dermal fibroblasts (hDFbs)

hDFbs were isolated from skin samples discarded from abdominoplasty surgeries of consenting patients at Hospital da Prelada (Porto, Portugal). Briefly, the skin tissue was cut in pieces of 0.5 by 0.5 cm and incubated in a dispase solution (2.4 $U.mL^{-1}$ in PBS) at 4 °C overnight. After the tissue digestion, the epidermis was separated from the dermis, and the dermal pieces were digested overnight in a collagenase IA solution (125 $U.mL^{-1}$ in PBS) at 4 °C. Digestion products containing hDFbs were poured through a 100 µm cell strainer and centrifuged at 200 *g* for 5 minutes. The pellet was resuspended and the fibroblasts were subsequently cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma, Germany) supplemented with 10% of fetal bovine serum (FBS, Gibco, UK) and 1% (v/v) antibiotic/antimycotic solution (A/B) (Gibco, UK) containing 100 $units.mL^{-1}$ penicillin and 100 $mg.mL^{-1}$ streptomycin, in a 37 °C humidified atmosphere with 5% CO_2.

Culture of hDFbs on the PA-HA membranes

Membranes were prepared under sterile conditions as previously described. HA sterilization for cell studies was done by dissolving the polymer in water, filtering the solution through a 0.22 µm filter, followed by lyophilization in sterile falcon tubes (Sartorius, USA). PA solutions were sterilized by UV exposure for 15 minutes. Confluent hDFbs were harvested from monolayer cultures using TrypLE™ Express (Invitrogen, USA). Cells were seeded on the membranes (10^4 cells.cm^{-2}) and cultured in DMEM supplemented with 10% FBS and 1% (v/v) A/B at 37 °C in a humidified atmosphere of 5% CO_2 for 7 days. For inhibition studies, the cell culture media was supplemented with 20 µM of GM6001 MMP-1 inhibitor (Chemicon, USA). Cell culture media was replaced twice a week, and the collected conditioned media was stored frozen (-80 °C) until further analysis. At predetermined time points, cell-cultured membranes were collected to assess cell proliferation (DNA quantification), morphology and spreading (SEM, F-actin staining) and production of MMP-1, collagen and ECM proteins. Cell culture experiments were performed in triplicate for each condition and time point.

Cell proliferation

Cell proliferation was assessed at different culture times (1, 3 and 7 days) by quantifying the amount of double-stranded DNA (dsDNA). Quantification was performed using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Molecular Probes, Oregon, USA), according to the instructions of the manufacturer. Briefly, cells on the different membranes/coverslips were lysed by osmotic and thermal shock and the supernatant used for the DNA quantification. The fluorescent intensity of the dye was measured in a microplate reader (Synergie HT, Bio-Tek, USA) with excitation at 485/20 nm and emission at 528/20 nm. The DNA concentration for each sample was calculated using a standard curve (DNA concentration ranging from 0 to 1.5 mg/mL) relating

quantity of DNA and fluorescence intensity. Three samples were analyzed per condition and time point.

Staining and confocal microscopy

For examination of cell morphology and MMP-1 production at the protein level, fibroblasts cultured on the different membranes/coverslips were stained for F-actin and MMP-1. Cells were washed in PBS and subsequently fixed in 10% formalin solution (Sigma-Aldrich, Germany) for 30 minutes at 4 °C. Cells were then washed once with 0.1 M glycine in PBS and twice with PBS and permeabilized with 2% BSA/ 0.2% Triton X-100 solution for 1 h at RT. Samples were incubated with primary antibody anti-MMP-1 (ab38929, 1:100, abcam, UK) for 1 h at RT and washed three times for 2 minutes with PBS. Samples were then incubated with the secondary antibody, anti-mouse Alexa 488 (1:200, Molecular Probes, Invitrogen, USA) and TRITC-conjugated phalloidin (1 U/mL, Sigma-Aldrich, Germany) for 1 h at RT. Cell nuclei were counterstained with 1 mg/mL DAPI (1:1000, Sigma-Aldrich, Germany) for 10 min and washed with PBS. Visualization was performed by confocal laser scanning microscopy (CLSM, Olympus FluoView 1000, Olympus, Japan). Background was subtracted and images were processed using FV10-ASW 3.1 software (Olympus, Japan).

Expression of MMP-1

MMP-1 expression by hDFbs was evaluated by analyzing the conditioned medium collected from fibroblast cultures on the PA-HA membranes, or peptide-coated glass coverslips, at different culture times (1, 3 and 7 days). MMP-1 was quantified with a double sandwich enzyme-linked Immunosorbent assay (ELISA) kit (Human Total MMP-1 DuoSet, R&D Systems, USA) according to the instructions of the manufacturer. Optical density (OD) was read at 450 nm and 570 nm in a microplate reader (Synergie HT, Bio-Tek, USA). The MMP-1 concentration for each sample was

calculated using a standard curve relating quantity of MMP-1 and OD. Three samples were analyzed per condition and time point.

Quantification of collagen and total ECM proteins

The deposition of ECM proteins by hDFbs cultured on the different membranes was analysed by quantification of collagen and other non-collagenous proteins using a colorimetric analysis (Sirius red/Fast green collagen staining kit, Chondrex, USA) and following the instructions of the manufacturer.[50] Briefly, cells were washed in PBS and subsequently fixed in 10% formalin solution (Sigma-Aldrich, Germany) for 30 minutes at 4 °C. Cells were washed again in PBS and immersed in 200 µL of the dye solution containing Sirius red and fast green. The plates were incubated at RT for 30 minutes in a rotary shaker. The dye solutions were then carefully withdrawn, and the plates were washed repeatedly with distilled water until the solution was colorless. After washing, 1 mL of dye extraction solution was added to each sample to elute the color. Optical density (OD) was read at 540 nm and 605 nm in a microplate reader (Synergie HT, Bio-Tek, USA). The values of collagen and total ECM proteins were calculated following the manufacturer instructions and normalized to DNA content.

Data analysis and statistics

All data values are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 5.00 software (San Diego, USA). Statistical differences between the different conditions (NAG, DNA, MMP-1 and collagenous/non-collagenous protein quantifications) were determined using a two-way analysis of variance (ANOVA) with a Bonferroni's multiple comparison post-hoc test (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

References

[1] M. Zelzer, S. J. Todd, A. R. Hirst, T. O. McDonald, R. V. Ulijn, Biomaterials Science 2013, 1, 11.

[2] M. P. Lutolf, J. L. Lauer-Fields, H. G. Schmoekel, A. T. Metters, F. E. Weber, G. B. Fields, J. A. Hubbell, Proceedings of the National Academy of Sciences 2003, 100, 5413.

[3] H. G. Schmoekel, F. E. Weber, J. C. Schense, K. W. Gratz, P. Schawalder, J. A. Hubbell, Biotechnol Bioeng 2005, 89, 253; A. H. Zisch, U. Schenk, J. C. Schense, S. E. Sakiyama-Elbert, J. A. Hubbell, J Control Release 2001, 72, 101.

[4] K. B. Fonseca, F. R. Maia, F. A. Cruz, D. Andrade, M. A. Juliano, P. L. Granja, C. C. Barrias, Soft Matter 2013, 9, 3283.

[5] S. Khetan, J. S. Katz, J. A. Burdick, Soft Matter 2009, 5, 1601.

[6] S. Kim, K. E. Healy, Biomacromolecules 2003, 4, 1214; N. S. Khelfallah, G. Decher, P. J. Mésini, Macromolecular Rapid Communications 2006, 27, 1004.

[7] P.-F. Caponi, X.-P. Qiu, F. Vilela, F. M. Winnik, R. V. Ulijn, Polymer Chemistry 2011, 2, 306; J. H. Collier, P. B. Messersmith, Bioconjugate Chemistry 2003, 14, 748; Z. Yang, M. Ma, B. Xu, Soft Matter 2009, 5, 2546; D. Koda, T. Maruyama, N. Minakuchi, K. Nakashima, M. Goto, Chemical Communications 2010, 46, 979.

[8] H. W. Jun, V. Yuwono, S. E. Paramonov, J. D. Hartgerink, Advanced Materials 2005, 17, 2612; M. C. Giano, D. J. Pochan, J. P. Schneider, Biomaterials 2011, 32, 6471.

[9] K. M. Galler, L. Aulisa, K. R. Regan, R. N. D'Souza, J. D. Hartgerink, J Am Chem Soc 2010, 132, 3217.

[10] Y. Chau, Y. Luo, A. C. Y. Cheung, Y. Nagai, S. Zhang, J. B. Kobler, S. M. Zeitels, R. Langer, Biomaterials 2008, 29, 1713.

[11] M. J. Webber, C. J. Newcomb, R. Bitton, S. I. Stupp, Soft Matter 2011, 7, 9665.

[12] A. Dehsorkhi, I. W. Hamley, J. Seitsonen, J. Ruokolainen, Langmuir 2013, 29, 6665.

[13] Y.-A. Lin, Y.-C. Ou, A. G. Cheetham, H. Cui, Biomacromolecules 2014, 15, 1419.

[14] T. H. Vu, Z. Werb, Genes & Development 2000, 14, 2123; A. Page-McCaw, A. J. Ewald, Z. Werb, Nat Rev Mol Cell Biol 2007, 8, 221.

[15] V.-M. Kähäri, U. Saarialho-Kere, Experimental Dermatology 1997, 6, 199.

[16] S. V. Nolte, W. Xu, H. O. Rennekampff, H. P. Rodemann, Cells Tissues Organs 2008, 187, 165.

[17] M. P. Lutolf, G. P. Raeber, A. H. Zisch, N. Tirelli, J. A. Hubbell, Advanced Materials 2003, 15, 888.

[18] S. Werner, R. Grose, Physiol Rev 2003, 83, 835.

[19] J. L. West, J. A. Hubbell, Macromolecules 1998, 32, 241.

[20] J. Patterson, J. A. Hubbell, Biomaterials 2010, 31, 7836.

[21] K. Bott, Z. Upton, K. Schrobback, M. Ehrbar, J. A. Hubbell, M. P. Lutolf, S. C. Rizzi, Biomaterials 2010, 31, 8454.

[22] K. M. Galler, J. D. Hartgerink, A. C. Cavender, G. Schmalz, R. N. D'Souza, Tissue Eng Part A 2012, 18, 176.

[23] J.-K. Kim, J. Anderson, H.-W. Jun, M. A. Repka, S. Jo, Molecular Pharmaceutics 2009, 6, 978; J. Banerjee, A. J. Hanson, B. Gadam, A. I. Elegbede, S. Tobwala, B. Ganguly, A. V. Wagh, W. W. Muhonen, B. Law, J. B. Shabb, D. K. Srivastava, S. Mallik, Bioconjug Chem 2009, 20, 1332.

[24] J. Banerjee, A. J. Hanson, E. K. Nyren-Erickson, B. Ganguli, A. Wagh, W. W. Muhonen, B. Law, J. B. Shabb, D. K. Srivastava, S. Mallik, Chem Commun (Camb) 2010, 46, 3209.

[25] K. T. Dicker, L. A. Gurski, S. Pradhan-Bhatt, R. L. Witt, M. C. Farach-Carson, X. Jia, Acta Biomaterialia 2014, 10, 1558; J. A. Burdick, G. D. Prestwich, Advanced Materials 2011, 23, H41.

[26] A. Almond, Cellular and Molecular Life Sciences 2007, 64, 1591; T. C. Laurent, J. R. E. Fraser, Faseb Journal 1992, 6, 2397.

[27] B. P. Toole, Nature Reviews Cancer 2004, 4, 528; M. A. Solis, Y.-H. Chen, T. Y. Wong, V. Z. Bittencourt, Y.-C. Lin, L. L. H. Huang, Biochemistry research international 2012, 2012, 346972.

[28] M. David-Raoudi, F. Tranchepain, B. Deschrevel, J. C. Vincent, P. Bogdanowicz, K. Boumediene, J. P. Pujol, Wound Repair Regen 2008, 16, 274; R. A. Clark, K. Ghosh, M. G. Tonnesen, J Invest Dermatol 2007, 127, 1018; W. Y. Chen, G. Abatangelo, Wound Repair Regen 1999, 7, 79.

[29] R. M. Capito, H. S. Azevedo, Y. S. Velichko, A. Mata, S. I. Stupp, Science 2008, 319, 1812.

[30] D. S. Ferreira, A. P. Marques, R. L. Reis, H. S. Azevedo, Biomaterials Science 2013, 1, 952.

[31] A. C. Mendes, K. H. Smith, E. Tejeda-Montes, E. Engel, R. L. Reis, H. S. Azevedo, A. Mata, Advanced Functional Materials 2013, 23, 430.
[32] J. D. Hartgerink, E. Beniash, S. I. Stupp, Science 2001, 294, 1684.
[33] M. Ehrbar, S. C. Rizzi, R. G. Schoenmakers, B. S. Miguel, J. A. Hubbell, F. E. Weber, M. P. Lutolf, Biomacromolecules 2007, 8, 3000.
[34] R. T. Aimes, J. P. Quigley, Journal of Biological Chemistry 1995, 270, 5872.
[35] H. Nagase, G. B. Fields, Biopolymers 1996, 40, 399.
[36] W. Babel, R. W. Glanville, European Journal of Biochemistry 1984, 143, 545.
[37] N. Greenfield, G. D. Fasman, Biochemistry 1969, 8, 4108.
[38] H. A. Behanna, J. J. Donners, A. C. Gordon, S. I. Stupp, J Am Chem Soc 2005, 127, 1193.
[39] A. C. Mendes, E. T. Baran, R. L. Reis, H. S. Azevedo, Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology 2013, 5, 582.
[40] R. Langer, D. A. Tirrell, Nature 2004, 428, 487; L. G. Griffith, M. A. Swartz, Nat Rev Mol Cell Biol 2006, 7, 211; M. P. Lutolf, J. A. Hubbell, Nature Biotechnology 2005, 23, 47.
[41] T. J. Shaw, P. Martin, Journal of Cell Science 2009, 122, 3209.
[42] M. J. Webber, J. Tongers, M. A. Renault, J. G. Roncalli, D. W. Losordo, S. I. Stupp, Acta Biomaterialia 2010, 6, 3.
[43] B. Martin-Martin, V. Tovell, A. H. Dahlmann-Noor, P. T. Khaw, M. Bailly, Eur J Cell Biol 2011, 90, 26.
[44] Z. Werb, Cell 1997, 91, 439.
[45] A. D. Widgerow, Wound Repair Regen 2011, 19, 117.
[46] S. Werner, T. Krieg, H. Smola, J Invest Dermatol 2007, 127, 998; B. Hinz, J Invest Dermatol 2007, 127, 526.
[47] O. Langholz, D. Rockel, C. Mauch, E. Kozlowska, I. Bank, T. Krieg, B. Eckes, J Cell Biol 1995, 131, 1903.
[48] J. Varani, Y. Hattori, Y. Chi, T. Schmidt, P. Perone, M. E. Zeigler, D. J. Fader, T. M. Johnson, Br J Cancer 2000, 82, 657.
[49] T. Takahashi, M. Ikegami-Kawai, R. Okuda, K. Suzuki, Anal Biochem 2003, 322, 257.
[50] A. López-De León, M. Rojkind, Journal of Histochemistry & Cytochemistry 1985, 33, 737.

Figure 1. Design and characterization of the peptide amphiphiles (PAs) used in this study. (A) Chemical structure of the PAs showing the different functional segments. (B) Circular dichroism spectra of PA solutions (0.011 mM) at pH 3, 7, 9. (C) TEM images of PA nanostructures negatively stained with uranyl acetate (nanofibers were formed by the deposition of 0.1 wt% solutions in water followed by air drying on a carbon-coated TEM grid).

Figure 2. Schematic illustration of the proposed enzyme-mediated degradation of self-assembled membranes made of hyaluronan (HA) and peptide nanofibers containing a cleavable site for MMP-1. The incorporation of a MMP-sensitive domain into the membrane design is expected to enhance their degradation by fibroblast secreted enzymes (HAase, MMP-1) and this would lead to enhance cellular invasion.

Figure 3. Degradation profile of the self-assembled membranes containing different functionalities. (A) Quantification of N-acetylamino sugars released from the membranes when incubated in PBS and in PBS containing 50 U/mL HAase, 10 nM human MMP-1 or both enzymes; Control (K_3-HA, A1), MMP sensitive (MMP_sK_3-HA, A2) and MMP insensitive (MMP_iK_3-HA, A3) (*p< 0.05, error bars represent standard deviation (n=3)). (B1) SEM images showing differences in membrane microstructure when exposed to enzyme solutions up to 14 days. (B2) Cross section of the MMP_sK_3-HA membranes after exposure to 10 nM MMP-1.

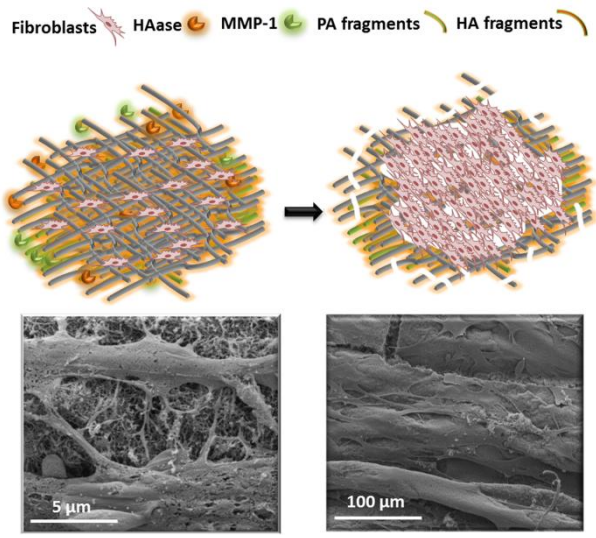
Figure 4. Fibroblast proliferation when cultured on membranes with different functionalities. hDFb proliferation assessed by dsDNA quantification. (*p< 0.05, error bars represent standard deviation (n= 3)).

Figure 5. Effect of membrane functionalization on the expression of MMP-1 by fibroblasts (hDFbs). (A) ELISA quantification of MMP-1 in cell culture supernatants (*p< 0.05, error bars represent standard deviation (n= 3)). (B) Confocal microscopy images of fibroblasts stained for MMP-1 (green), F-actin (red) and nuclei (blue) after 24 hours.

Figure 6. Concentration of ECM proteins, collagenous (A) and non-collagenous (B), secreted by hDFbs when cultured on the membranes with different functionalities (*p< 0.05, error bars represent standard deviation (n= 3)).

Figure 7. SEM micrographs showing differences in membrane microstructure after culturing hDFbs for 14 days, suggesting membrane cell-mediated degradation.

Table of contents



Self-assembling membranes, molecularly designed with enzyme-cleavable building blocks (hyaluronan and peptide amphiphiles containing a proteolytic domain) afford cell-mediated degradation and leads to enhanced cellular colonization of the membranes. This concept can be used as a strategy to develop artificial matrices with more biomimetic degradation for tissue engineering applications.

Supporting Information

Click here to download Supporting Information: adhm 201400586_Revised_SI_final.docx

Figure 1
Click here to download Production Data: Figure 1.tif

Figure 2
Click here to download Production Data: Figure 2.tif

Figure 3
Click here to download Production Data: Figure 3.tif

Figure 4
Click here to download Production Data: Figure 4.tif

Figure 5
Click here to download Production Data: Figure 5.tif

Figure 6
Click here to download Production Data: Figure 6.tif

Figure 7
Click here to download Production Data: Figure7.tif

Image for Table of Contents

Click here to download Production Data: GraphicalAbstract.tif