Biochemical and Biophysical Research Communications 459 (2015) 246–251

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Proteolysis of decellularized extracellular matrices results in loss of fibronectin and cell binding activity

Anand Ramanathan, Nancy Karuri*

Department of Chemical and Biological Engineering, Illinois Institute of Technology, 10 W. 33rd St, Chicago, IL 60616, USA

ARTICLE INFO

Article history:
Received 3 February 2015
Available online 25 February 2015

Keywords:
Fibronectin
Decellularized extracellular matrix
Proteolysis
Cell adhesion

ABSTRACT

Excessive inflammation in the chronic wound bed is believed to result in increased fibronectin (FN) proteolysis and poor tissue repair. However, FN fragments can prime the immune response and result in higher protease levels. The reciprocity between FN proteolysis and inflammation makes it challenging to determine the specific contribution of FN proteolysis in the extracellular matrix (ECM) on tissue responses. We studied the impact of proteolysis of decellularized extracellular matrices (dECMs) obtained from NIH 3T3 mouse fibroblasts on FN level and activity. The dECMs were treated with α chymotrypsin and proteolysis was stopped at different time points. The protease solution was obtained, the remaining dECM was scrapped and examined by immunoblotting and Bicinchoninic Acid assays. Fibronectin was 9.4 ± 1.8% of the total protein content in the dECM but was more susceptible to proteolysis. After 15 min of protease treatment there was a 67.6% and 11.1% decrease in FN and total protein, respectively, in the dECMs. Fibronectin fragments were present both in the proteolysis solution and in the dECM. Cell adhesion, spreading and actin extensions on dECMs decreased with increasing proteolysis time. Interestingly, the solutions obtained after proteolysis of the dECMs supported cell adhesion and spreading in a time dependent manner, thus demonstrating the presence of FN cell binding activity in the protease solution of dECMs. This study demonstrates the susceptibility of FN in the ECM to proteolysis and the resulting loss of cell adhesion due to the decrease of FN activity and places weight on bioengineering strategies to stabilize FN against proteolysis.

1. Introduction

Fibronectin (FN) is a key component of the extracellular matrix (ECM). It is a 270 kDa dimeric protein and consists of functional domains that mediate interactions with cells and other extracellular molecules (Fig. 1A). These interactions serve to attract and attach different cells and molecules to the wound bed during healing [1,2]. Abnormally high levels of FN fragmentation have been linked to elevated levels of inflammation proteases in chronic wounds [3–5]. FN fragments can also stimulate the immune system [6–8], thereby exacerbating FN proteolysis. Teasing out the contribution of FN proteolysis on tissue response with a chronic wound model is challenging due to the reciprocity between inflammation and FN proteolysis. Moreover, there are other ECM molecules in the wound bed that mediate responses associated with tissue repair [9–11] and that are susceptible to proteolysis. Therefore, the importance of FN proteolysis relative to other proteins in the ECM needs to be delineated.

The goal of this study was to determine the effect of protease on the FN levels and cell binding activity in a decellularized extracellular matrix (dECM). We focused specifically on cell adhesion and spreading on a dECM because these responses are fundamental to FN matrix assembly [1,12] and wound repair [10]. We demonstrate that FN is more susceptible to proteolytic fragmentation in dECMs than total ECM protein content. We report a link between FN fragmentation in dECMs after protease treatment and loss of cell adhesion and spreading. The studies support the idea of stabilizing FN against proteolysis as a potential therapeutic strategy for mitigating loss of ECM function in a high protease environment.

2. Materials and methods

2.1. dECM preparation

We used a protocol based on the method by Castello-Cros et al. [13] to isolate dECMs from NIH 3T3 mouse fibroblast culture. Glass

Abbreviations: FN, fibronectin; ECM, extracellular matrix; dECM, decellularized extracellular matrix.

* Corresponding author. Fax: +1 312 567 8874.
E-mail address: nkaruri1@iit.edu (N. Karuri).

http://dx.doi.org/10.1016/j.bbrc.2015.02.092
0006-291X/

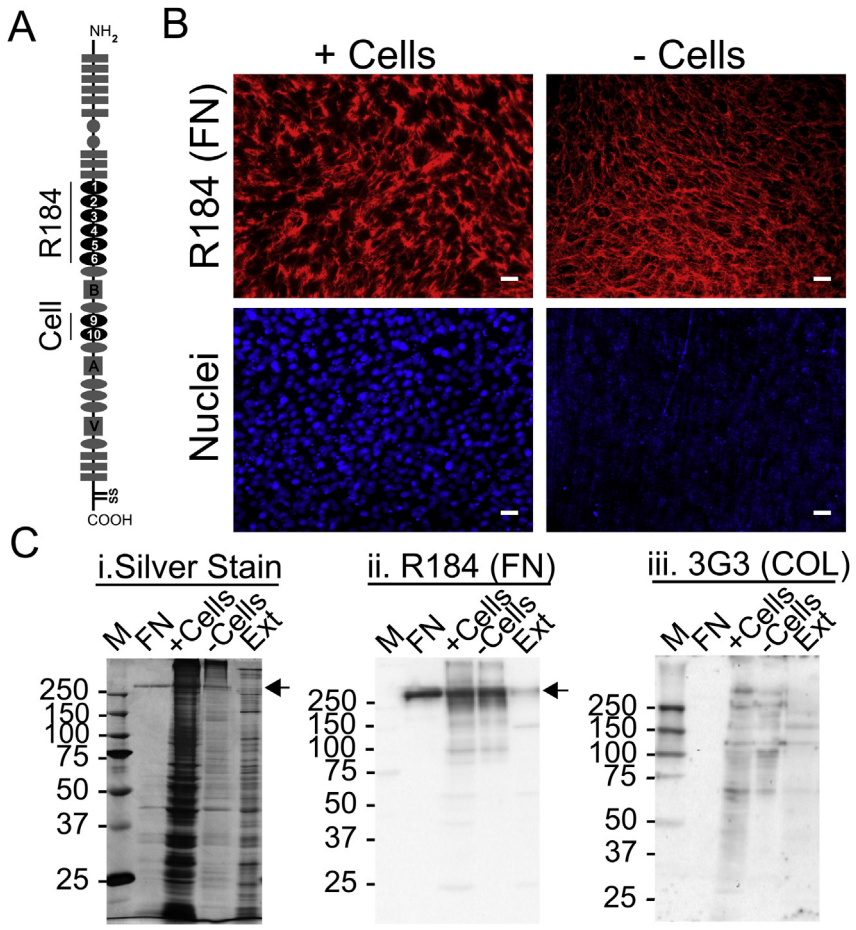

Fig. 1. Cell extraction does not deplete FN in dECMs. (A) FN is composed of homologous structural repeats classified as type I (rectangles), II (circles) and III (ovals) [1]. R184 antibodies against FN used in immunoblotting and fluorescence microscopy bind to repeats $III_{1\text{-}6}$ (labeled R184). $III_{9\text{-}10}$ (labeled Cell) mediates cell adhesion through interactions with integrins. (B) Fluorescence microscopy images of FN (rhodamine conjugated R184) and cell nuclei (Hoechst 33258) in cultured layers of NIH 3T3 mouse fibroblasts before (+Cells) and after (-Cells) cell extraction. Scale bar is = 50 μm. (C) SDS-PAGE analysis of reduced ECM with cells, without cells and extraction buffer (labeled + Cells, -Cells and Ext) followed by (i) silver staining or (ii) R184 immunoblotting or (iii) 3G3 immunoblotting against collagen I. M = molecular weight standards. Lane marked FN represents 100 ng human plasma FN loading control. Arrows mark molecular weight of reduced FN.

cover slips (12 mm) were incubated in 0.2% gelatin (Fisher Scientific, Pittsburgh, PA) in phosphate buffered saline (PBS, Fisher Scientific), overnight at 4 °C. Gelatin was crosslinked with 1% glutaraldehyde (Sigma Aldrich, St. Louis, MO) in PBS for 30 min at room temperature. Glutaraldehyde was quenched with 1 M Trisamine-HCl (Tris–HCl, Fisher Scientific), pH 8.8 for 30 min at room temperature. NIH 3T3 mouse fibroblasts (ATCC, Manasses, VA) were seeded at 1×10^5 cells per coverslip in Dulbecco's Modified Eagle's Medium (DMEM, Fisher Scientific) supplemented with bovine calf serum (Fisher Scientific). The cells were cultured in 24 well dishes for 24 h at 37 °C in 5% CO_2. Cell culture medium was refreshed with complete medium supplemented with 50 μg/ml ascorbic acid (Sigma Aldrich) and culture was then maintained for an additional 48 h. This step was repeated, for a total culture time of 120 h. Cells were extracted from the ECM by incubating the coverslips with 0.5% Triton (Sigma Aldrich), 20 mM NH_4OH (Sigma Aldrich) in PBS for 10 min at 37 °C. The dECMs were washed with three changes of PBS and then stored in PBS at 4 °C for up to a month. Cell extraction was confirmed by fluorescence microscopy of nuclei.

2.2. Proteolysis and characterization of proteolyzed dECMs

The dECMs were incubated with 5 μg/ml α chymotrypsin (Sigma Aldrich) in 100 mM Tris–HCl and 10 mM $CaCl_2$, pH 7.8. Proteolysis was stopped at 0, 5 and 15 min with 2 mM phenylmethylsulfonyl fluoride (PMSF, Sigma Aldrich). The protease solution was collected and the dECMs were washed in PBS and solubilized in 1% sodium dodecyl sulfate (SDS, Fisher Scientific) in 25 mM Tris–HCl, pH 8.0. The protease solution and solubilized dECM samples were prepared for electrophoresis by the addition of an electrophoresis buffer containing SDS, glycerol (Fisher Scientific) and dithioreitol (Fisher Scientific) to a final solution concentration of 1.33%, 6.6% and 0.07 M, respectively. The samples were boiled and resolved on 5 or 10% polyacrylamide gels and stained with silver nitrate or transferred to a nitrocellulose membrane (GE Healthcare Bio-Sciences, Pittsburgh, PA). The membrane was probed with rabbit polyclonal antibody R184 against FN domains $III_{1\text{-}6}$ (Fig. 1A) or mouse monoclonal antibody 3G3 (Abcam, Cambridge, MA) against collagen I followed by goat anti-rabbit IgG (H + L) horseradish peroxidase (Invitrogen, Eugene, OR) or goat anti-mouse IgG (H + L) horseradish peroxidase (Invitrogen), respectively. The blots were treated with Pierce ECL western blotting substrate (Fischer Scientific) and imaged in a ChemiDoc XRS + imaging system (BioRad, Hercules, CA). Densitometric analyses were carried out with Image Lab software (BioRad). A loading standard of 100 ng human plasma FN was used to normalize raw intensity. Human plasma FN was obtained using established protocols [14,15]. The total protein amount in the protease solutions and solubilized ECMs was measured by Bicinchoninic Acid (BCA) assay.

2.3. Cell adhesion assay

NIH 3T3 mouse fibroblasts passages 4–12 were used for cell adhesion assays. The assays were carried out on dECMs or coverslips coated with the solutions obtained after proteolysis of dECMs at different time points. Cell adhesion assays were carried out in serum free conditions using previously reported protocols [14,16–19]. The seeding density was 1×10^5 cells per dECM or coverslip. Actin, nuclei and FN were stained with fluorescein conjugated phalloidin (Invitrogen), Hoechst 33258 (Fisher Scientific) and R184 antibodies against FN, respectively. Goat anti-rabbit IgG (H + L) conjugated to rhodamine (Invitrogen) was used to visualize R184 localization.

2.4. Microscopy

Imaging was carried out at 10 and 20× magnifications using a Carl Zeiss Axiovert 200M microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY) coupled to an Axiocam MRm camera (Carl Zeiss Microscopy) to determine number of attached cells and cell spreading area, respectively. Cell number and spreading area were quantified using Image J software (National Institute of Health). For higher resolution, a Zeiss LSM 5 PASCAL confocal microscope (Carl Zeiss Microscopy) with a 63× oil objective, and low pass filter of 505 nm and an excitation of 488 nm was used to image fluorescein stained actin. A 560 nm low pass filter and an excitation of 543 nm was used to image rhodamine stained FN. Pinhole size and detector gain were maintained constant when imaging all treatment types. Actin extensions were considered to be any protrusion from the cell body that was half of the cell body diameter or greater.

2.5. Statistical analysis

All experiments were conducted at least two times, with two replications per treatment. A one-factor variance was used to evaluate the effect of each treatment on cell attachment and spreading. P-values less than or equal to 0.05 were considered statistically significant. All uncertainties are reported as the 95% confidence interval of the mean.

3. Results

3.1. Presence of fibrillar FN in dECMs

Decellularized ECMs were isolated from NIH 3T3 mouse fibroblasts cultured on gelatin coated glass coverslips. Fig. 1B shows fluorescence microscopy of the NIH 3T3 mouse fibroblast culture layer before and after cell extraction. FN immunofluorescence in the dECM was in the form of fibrillar structures (Fig. 1B, top panel). Qualitatively the intensity of FN immunofluorescence on the glass coverslips was comparable before and after cell extraction, indicating that FN in the ECM was not significantly perturbed by the cell extraction procedure. Positive staining for cell nuclei was not observed in dECMs (Fig. 1B, bottom panel), even with maximum imaging exposure, indicating the absence of cells.

Bicinchoninic Acid assays showed that detergent extraction of cells eliminated 80.9 ± 0.7% of the total proteins from the culture layer and this was confirmed by SDS-PAGE analysis (Fig. 1Ci). Immunoblotting with R184 antibodies against FN (Fig. 1Cii) showed the presence of intact FN and some FN fragmentation in the ECM before and after cell extraction. This was attributed to cell mediated proteolysis during ECM remodeling. Densitometric analysis of the blots showed that 82.0 ± 8.3% of the total FN was retained after cell extraction. Collagen I, a major ECM component, was not significantly perturbed by cell extraction (Fig. 1Ciii). The extraction procedure was effective at generating dECMs without significantly perturbing FN content.

3.2. Protease treatment of dECMs results in significant FN proteolysis

The effect of protease on FN in the ECM was determined by adding α chymotrypsin to the dECMs and analyzing the products of proteolysis at different time points. Protease treatment of dECMs resulted in ECM fragmentation and release of fragments into the protease solution (Fig. 2A). The decrease and increase in protein content in the dECMs and protease solutions, respectively, was confirmed by BCA analysis (Supplementary Fig. 1). The total protein of the dECM decreased by 11.1 ± 7.4% following 15 min of protease treatment. The fragmented proteins that were released from the dECM were identified by immunoblotting (Fig. 2B and C). Immunoblotting with R184 antibodies showed that FN was a substantial component of the dECM fragments released into the protease solution after 15 min (Fig. 2B). Immunoblotting with 3G3 antibodies against collagen I showed that it is neither fragmented in the dECM nor detected in the protease solution following protease treatment (Fig. 2C). Fibronectin in the dECM was quantified by densitometric analysis of R184 immunoblots and normalized by total protein in the dECM (Fig. 2D). The total amount of FN containing the epitope for R184 antibodies in dECMs decreased by 67.6 ± 17.6% after 15 min of protease treatment. While collagen I is a majority of the protein synthesized by fibroblasts [20], densitometric analysis did not detect a significant decrease in collagen I content following protease treatment. These results indicate that FN in the ECM is highly susceptible to proteolysis and a significant amount of FN is lost to solution during ECM proteolysis.

3.3. NIH 3T3 fibroblast adhesion on dECMs is decreased after protease treatment

Fibronectin mediates cell adhesion in the ECM through intermolecular interactions between the FN cell binding domain and cells [21–23]. Since FN was rapidly fragmented in the dECMs during protease treatment, we examined the ability of proteolyzed dECMs to support cell adhesion. Fibroblasts adhering to dECMs were oval shaped and formed multiple actin extensions (Fig. 3A). Fibroblasts also adhered to dECMs that had been treated with protease; however, these cells were circular in shape and formed fewer actin extensions (Fig. 3A). The number of fibroblasts adhering to the dECMs and their spreading area decreased with increased proteolysis time (Fig. 3B and C). Interestingly, the greatest decrease in cell number and cell area was detected in the first 5 min of proteolysis. The number of actin extensions formed per fibroblast decreased with increased proteolysis time (Fig. 3D). FN immunostaining with R184 antibodies did not differ significantly between 0 and 5 min proteolysis time points (Fig. 3A). After 15 min proteolysis, FN staining intensity was visually lower and FN fibrils were shorter and disjointed.

Colocalization of actin extensions with FN fibrils was analyzed by confocal microscopy (see Supplementary Fig. 2). Intensity profiles for actin and FN staining in dECMs were positively correlated, indicating that cell extensions follow FN fibrils. This behavior was consistent in dECMs at 0 and 5 min protease treatment. Collectively, these findings demonstrate that protease treatment of the dECM alters FN mediated cell binding, which results in decreased cell attachment, cell spreading and cell extensions.

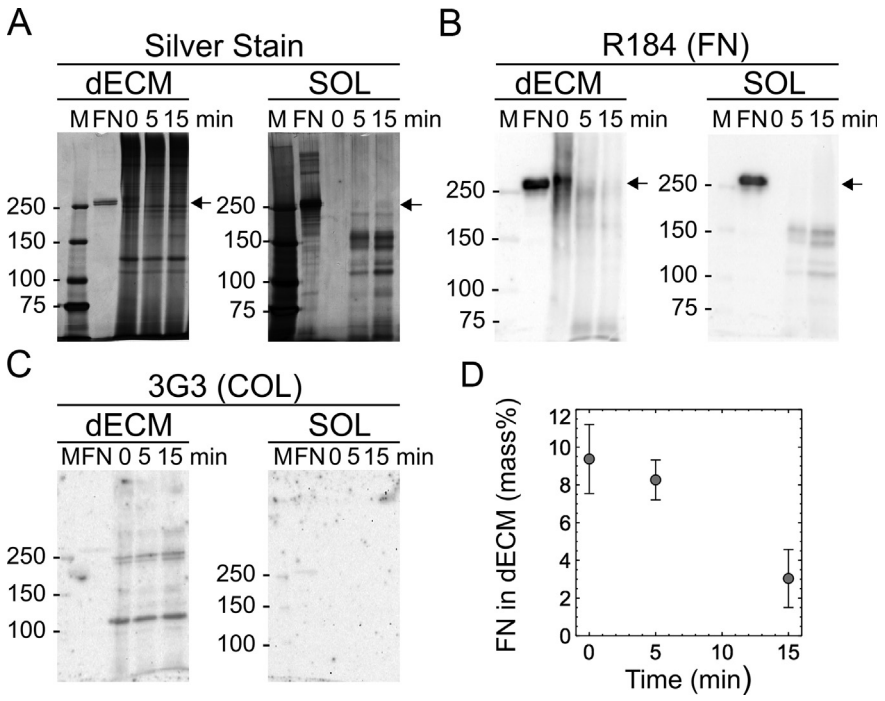

Fig. 2. FN is fragmented and released from the dECM following treatment with α chymotrypsin. (A) 5% silver stained polyacrylamide gels of solubilized dECM after treatment with α chymotrypsin for 0, 5 and 15 min 'SOL' represents the protease solutions. (B, C) Immunoblots of A with R184 and 3G3 antibodies against FN and collagen I (COL), respectively. M = molecular weight standards. Lane marked FN represents 100 ng human plasma FN loading control. Arrows mark molecular weight of reduced FN. (D) Densitometric analysis of total FN binding in dECMs from R184 immunoblots. Optical density data has been normalized by total protein. Error bars represent a 95% confidence interval of the mean.

3.4. Fragments from proteolysis of dECMs support cell adhesion

Since FN fragments were released from the dECM during protease treatment, the resulting protease solutions were tested to determine if they supported cell adhesion. Surfaces coated with the protease solution containing proteolytic fragments of the dECM supported fibroblast adhesion and spreading (Fig. 4A). Cell attachment, spreading area and aspect ratio positively correlated to

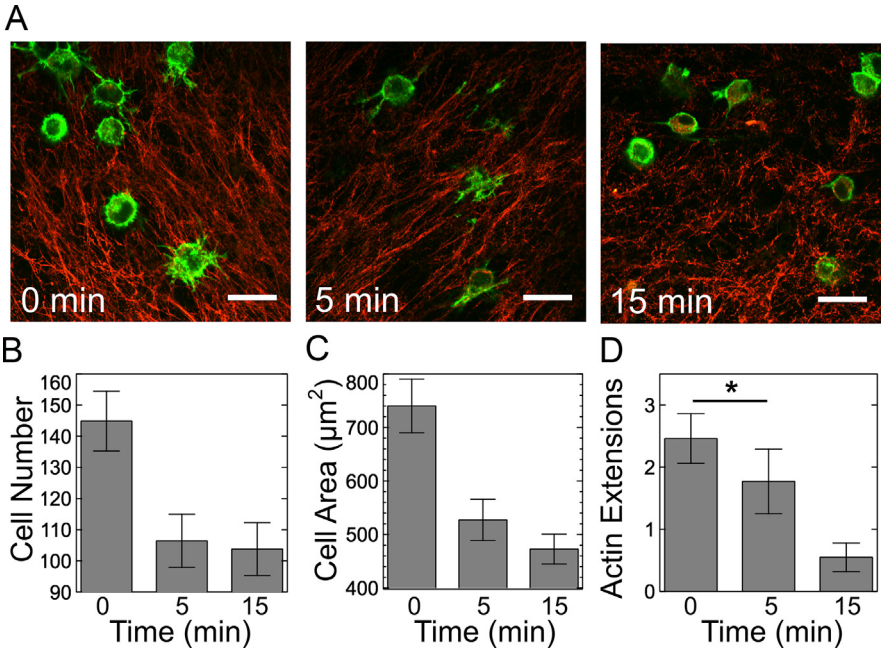

Fig. 3. Fibroblast adhesion on dECMs decreases with increased time of proteolysis. (A) Confocal microscopy images of NIH 3T3 mouse fibroblasts cultured on dECMs after treatment with α chymotrypsin for 0, 5 and 15 min. Green and red stain represents actin and FN respectively. Scale bar = 25 μm. The zero time point represents dECM before the addition of α chymotrypsin. (B, C) Cell number per 10× imaging frame and cell area under 20× objective on dECMs at different time points after protease treatment. (D) Number of actin extensions per cell, on dECMs at different time points after protease treatment. Error bars represent a 95% confidence interval of the mean. "*" represents a statistically significant difference in the mean. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

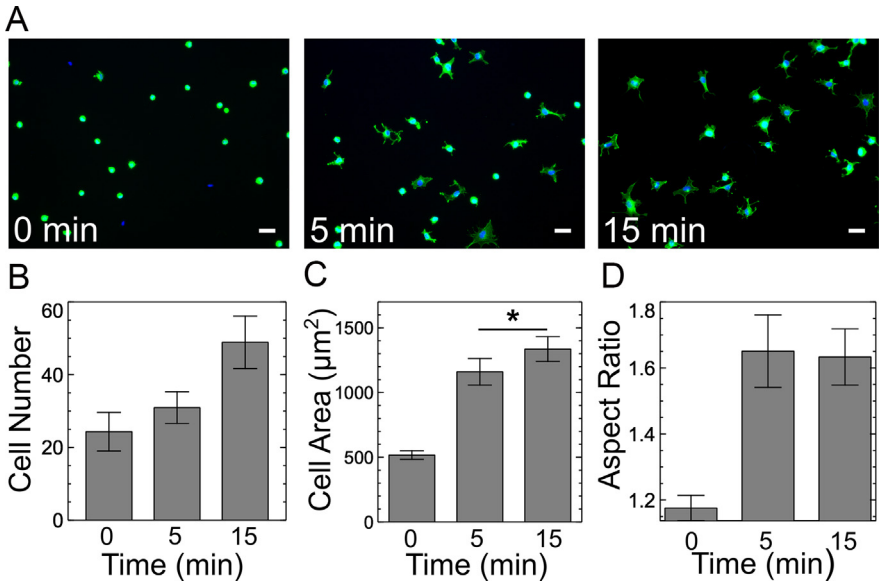

Fig. 4. Fibroblast adhesion on surfaces coated with proteolytic fragments from dECMs. (A) Fluorescence microscopy of NIH 3T3 mouse fibroblasts seeded on surfaces coated with solutions from dECMs collected after 0, 5 and 15 min proteolysis. Scale bar = 50 μm. (B, C) Cell number per 10× objective imaging frame and cell area under 20× objective of the samples in A. (D) Aspect ratio of cells under 20× objective of the samples in A. Error bars represent a 95% confidence interval of the mean. "*" represents a statistically significant difference in the mean.

proteolysis time (Fig. 4B, C and D). Actin extensions in cells cultured on the glass surfaces coated with protease solutions were visually shorter than cells cultured on dECMs; there were no extensions with a length of half the diameter of the cell body or greater on the former. This is consistent with studies showing significant differences in cell spreading in two and three dimensional cultures [23,24]. Collectively the data demonstrates that protease treatment of the dECM results in the generation and release of FN fragments that support cell adhesion.

4. Discussion

The goal of this study was to determine the effect of protease treatment on the amount of FN in dECMs and correlate FN fragmentation to biological activity. Decellularized ECMs provide a well-defined environment where confounding contributions of cells and extracellular components found in chronic wound fluids are eliminated. Treatment of dECMs with α chymotrypsin resulted in FN fragmentation in a time dependent manner and the decrease of FN in the dECM was significantly greater than the decrease of total protein in the dECM. Protease treatment of dECMs also resulted in loss of cell adhesion and spreading, functions associated with interactions between FN and cells [23,25]. The proteolysis solutions of dECMs contained a significant amount of fragmented FN and supported cell adhesion in a manner that was dependent on the time of proteolysis. This is significant because the protease treated dECMs still contained FN fragments but had significantly lower cell adhesion and spreading than intact dECMs. These studies show that FN in a dECM is fragmented and lost in solution during proteolysis, leading to reduced cell attachment on the dECM.

The novelty of our study is that we demonstrate, for the first time, in a well-studied and well-defined system, higher FN proteolysis in the ECM compared to total protein and a robust link between FN proteolysis and loss of FN mediated activity. Simultaneous increases in FN fragmentation and protease expression in tissues has been demonstrated in mouse models for inflammation [26,27] and FN fragments from the fluid of inflamed periodontal tissue have been shown to contain the EIIIA domain [28], a domain found in tissue FN but not in plasma FN. Tissue FN is secreted and assembled by fibroblasts [2] and these findings suggest that high levels of inflammation would result in FN proteolysis in tissues. Wound fluid contains proteolytic FN fragments which support cell adhesion [29]. These past studies contain cells or soluble extracellular molecules, such as growth factors, that may confound the contributions of FN on biological activity in the ECM. We demonstrate in a cell free system that a substantial amount of FN is fragmented and lost from dECMs and this loss is related to reduced cell binding. Our observations are made in the absence of cells, soluble extracellular molecules and in a controlled proteolytic environment, making our study novel. These results indicate that FN stabilization within the ECM environment could be a therapeutic strategy for mitigating poor tissue repair in chronic wounds and a number of pathologies.

Conflict of interest

The authors have no conflict of interest.

Acknowledgments

The authors would like to thank Dr. Eric Brey for use of the confocal microscope, Dr. Ahmed Mirza for help on the acquisition of human plasma and Dr. Jean Schwarzbauer for the gift of R184 antibodies. This research is supported by start-up funds from the Illinois Institute of Technology to Nancy W. Karuri.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.092.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.092.

References

[1] R. Pankov, K.M. Yamada, Fibronectin at a glance, J. Cell. Sci. 115 (2002) 3861–3863.
[2] W.S. To, K.S. Midwood, Plasma and cellular fibronectin: distinct and independent functions during tissue repair, Fibrogenes. Tissue Repair 4 (2011) 21.
[3] F. Grinnell, M.F. Zhu, Fibronectin degradation in chronic wounds depends on the relative levels of elastase, α1-proteinase inhibitor, and α2-macroglobulin, J. Invest. Dermatol 106 (1996) 335–341.
[4] C.N. Rao, D.A. Ladin, Y.Y. Liu, K. Chilukuri, Z.Z. Hou, D.T. Woodley, α1-antitrypsin is degraded and non-functional in chronic wounds but intact and functional in acute wounds: the inhibitor protects fibronectin from degradation by chronic wound fluid enzymes, J. Invest. Dermatol 105 (1995) 572–578.
[5] Y.L. Kapila, S. Kapila, P.W. Johnson, Fibronectin and fibronectin fragments modulate the expression of proteinases and proteinase inhibitors in human periodontal ligament cells, Matrix Biol. 15 (1996) 251–261.
[6] K. Feghali, D. Grenier, Priming effect of fibronectin fragments on the macrophage inflammatory response: potential contribution to periodontitis, Inflammation 35 (2012) 1696–1705.
[7] B. Marom, M.A. Rahat, L. Weiss-Cerem, A. Kinarty, H. Bitterman, Native and fragmented fibronectin oppositely modulate monocyte secretion of MMP-9, J. Leukoc. Biol. 81 (2007) 1466–1476.
[8] D.H. Beezhold, C. Personius, Fibronectin fragments stimulate necrosis factor secretion by human monocytes, J. Leukoc. Biol. 51 (1992) 59–64.
[9] E.A. Wayner, W.G. Carter, R.S. Piotrowicz, T.J. Kunicki, The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoprotein Ic-IIa, J. Cell. Biol. 107 (1988) 1881–1891.
[10] M.P. Welch, G.F. Odland, R.A. Clark, Temporal relationships of F-actin bundle formation, collagen and fibronectin matrix assembly, and fibronectin receptor expression to wound contraction, J. Cell. Biol. 110 (1990) 133–145.
[11] E.A. Wayner, W.G. Carter, Identification of multiple cell adhesion receptors for collagen and fibronectin in human fibrosarcoma cells possessing unique α subunits and common β subunits, J. Cell. Biol. 105 (1987) 1873–1884.
[12] Y. Mao, J.E. Schwarzbauer, Fibronectin fibrillogenesis, a cell-mediated matrix assembly process, Matrix Biol. 24 (2005) 389–399.
[13] R. Castello-Cros, E. Cukierman, Stromagenesis during tumorigenesis: characterization of tumor-associated fibroblasts and stroma-derived 3D matrices, Methods Mol. Biol. 522 (2009) 275–305.
[14] C. Zhang, S. Hekmatfar, A. Ramanathan, N.W. Karuri, PEGylated human plasma fibronectin is proteolytically stable, supports cell adhesion, cell migration, focal adhesion assembly, and fibronectin fibrillogenesis, Biotechnol. Prog. 29 (2013) 493–504.
[15] A. Ramanathan, N. Karuri, Fibronectin alters the rate of formation and structure of the fibrin matrix, Biochem. Biophys. Res. Commun. 443 (2014) 395–399.
[16] C. Chiang, S.W. Karuri, P.P. Kshatriya, J. Schwartz, J.E. Schwarzbauer, N.W. Karuri, Surface derivatization strategy for combinatorial analysis of cell response to mixtures of protein domains, Langmuir 28 (2012) 548–556.
[17] P.P. Kshatriya, S.W. Karuri, C. Chiang, N.W. Karuri, A combinatorial approach for directing the amount of fibronectin fibrils assembled by cells that uses surfaces derivatized with mixtures of fibronectin and cell binding domains, Biotechnol. Prog. 28 (2012) 862–871.
[18] C. Zhang, S. Hekmatfer, N.W. Karuri, A comparative study of polyethylene glycol hydrogels derivatized with the RGD peptide and the cell-binding domain of fibronectin, J. Biomed. Mater. Res. A 102 (2013) 170–179.
[19] C. Zhang, R. Desai, V. Perez-Luna, N. Karuri, PEGylation of lysine residues improves the proteolytic stability of fibronectin while retaining biological activity, Biotechnol. J. 9 (2014) 1033–1043.
[20] R. Raghow, A.E. Postlethwaite, J. Keski-Oja, H.L. Moses, A.H. Kang, Transforming grow factor-β increases steady state levels of type I procollagen and fibronectin messenger RNAs posttranscriptionally in cultured human dermal fibroblasts, J. Clin. Invest 79 (1987) 1285–1288.
[21] G. Baneyx, L. Baugh, V. Vogel, Fibronectin extension and unfolding within cell matrix fibrils controlled by cytoskeletal tension, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 5139–5143.
[22] R. Pankov, E. Cukierman, B.Z. Katz, K. Matsumoto, D.C. Lin, S. Lin, C. Hahn, K.M. Yamada, Integrin dynamics and matrix assembly: tensin-dependent translocation of $\alpha_5\beta_1$ integrins promotes early fibronectin fibrillogenesis, J. Cell. Biol. 148 (2000) 1075–1090.
[23] E. Cukierman, R. Pankov, D.R. Stevens, K.M. Yamada, Taking cell-matrix adhesions to the third dimension, Science 294 (2001) 1708–1712.
[24] K.A. Beningo, M. Dembo, Y.L. Wang, Responses of fibroblasts to anchorage of dorsal extracellular matrix receptors, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 18024–18029.
[25] S.K. Akiyama, S.S. Yamada, W.T. Chen, K.M. Yamada, Analysis of fibronectin receptor function with monoclonal antibodies: roles in cell adhesion, migration, matrix assembly, and cytoskeletal organization, J. Cell. Biol. 109 (1989) 863–875.
[26] M. Kusubata, A. Hirota, T. Ebihara, K. Kuwaba, Y. Matsubara, T. Sasaki, M. Kusakabe, T. Tsukada, S. Irie, Y. Koyama, Spatiotemporal changes of fibronectin, tenascin-C, fibulin-1, and fibulin-2 in the skin during the development of chronic contact dermatitis, J. Invest. Dermatol 113 (1999) 906–912.
[27] E.D. Son, J.H. Shim, H. Choi, H. Kim, K.M. Lim, J.H. Chung, S.Y. Byun, T.R. Lee, Cathepsin G inhibitor prevents ultraviolet B-induced photoaging in hairless mice via inhibition of fibronectin fragmentation, Dermatology 224 (2012) 352–360.
[28] Q.H. Huynh, S. Wang, E. Tafolla, S.A. Gansky, S. Kapila, G.C. Armitage, Y.L. Kapila, Specific fibronectin fragments as markers of periodontal disease status, J. Periodontol. 73 (2002) 1101–1110.
[29] F. Grinnell, C.H. Ho, A. Wysocki, Degradation of fibronectin and vitronectin in chronic wound fluid: analysis by cell blotting, immunoblotting, and cell adhesion assay, J. Invest. Dermatol 98 (1992) 410–416.