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264159.40909091 1104966394 21687702.120482 2761167.1979167 2243959420 18411367700 12990422 38958027.5 44057321304 82603204219 5615281.55 9977415.7346939 7427247896 22190817744 14137124.686275

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Peptide Research 1990;4:95–101. Method to correlate tandem mass spectra of modified peptides to amino acid sequences in the protein database. Though species and tissue-specific differences may be contributory, human and bovine matrix protein sequence alignments reveal ≥ 85% homology in fibrillin-1 and chains of collagens I, III, IV, V and VI. Sun K, Park J, Gupta OT, Holland WL, Auerbach P, Zhang N, et al. Transl Res 2014;163:268–85. 56. Similarly, our past studies demonstrate the influence of endothelial matrices on smooth muscle behaviors [29,60], and of fibroblastic ECM components on endothelial shape and migration [61]. Front Immunol 2011;2:93. Indeed, the matrix-remodeling proteases produced by immune effector cells are necessary for removal of devitalized tissue and debris while serving to stimulate microvascular remodeling and angiogenic activation by promoting capillary sprout formation and endothelial cell migration [9,10]. Whether achieved through surgical (sharp) intervention, mechanical methods, autolysis, or application of exogenous enzymes, debridement not only allows clinicians to thoroughly assess wound severity, but likely aids in reducing bacterial burden, regulating inflammation, and removing obstructions or barriers to healing. Fibroblasts from non-healing human chronic wounds show decreased expression of beta ig-h3, a TGF-beta inducible protein. J Clin Invest 2007;117:1219–22. While the use of hydrogel devices and moisture-retaining dressings support immune cell-mediated clearance of necrotic tissue, these processes rely on the body's own immune system to mount a sufficient response to clear the necrotic tissue requiring several weeks and may require extended treatment periods to achieve complete debridement [21,22]. Briefly, HMVEC and HDF at 7–10 days post-confluence were washed three times with Tris-buffered saline (TBS, pH 7.2). pmid:19320882 8. pmid:19918148 53. Of extracellular matrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. Recombinant human FGF2 (R&D Systems, Minneapolis, MN), recombinant human PDGFbb (R&D Systems), and Santyl® collagenase (Smith & Nephew PLC, Fort Worth, TX) were used as positive controls for assays with HMVEC, HDF, and NHEK, respectively. Citation: Sheets AR, Demidova-Rice TN, Shi L, Ronfard V, Grover KV, Herman IM (2016) Identification and Characterization of Novel Matrix-Derived Bioactive Peptides: A Role for Collagenase from Santyl® Ointment in Post-Debridement Wound Healing? Inflammation and wound healing: the role of the macrophage. Endothelial sprouting and angiogenesis: matrix metalloproteinases in the lead. After thorough mixing, peptide-containing GFR Matrigel was placed in wells of 8-well chamber slides (BD Biosciences, San Jose, CA). Perhaps the non-overlapping nature stems from the possibility that the specific, collagenase-produced peptides are further cleaved by the non-specific proteases in the mixture, or vice versa, thereby producing the specific bioactive matrix fragments presently detected. Park J, Scherer PE. Interestingly, there is little overlap in the endothelial ECM peptides produced by purified Clostridial collagenase and those arising from Santyl® collagenase. The digested matrices were then mixed with reducing sample buffer containing a final concentration of 2% β-mercaptoethanol (Sigma-Aldrich), heated to 95°C for 5 minutes, separated by SDS-PAGE, and proteins were stained using the SilverQuest Silver Staining Kit (ThermoFisher Scientific, Waltham, MA). Briefly, growth factor-reduced (GFR) Matrigel (Corning Life Sciences, Tewksbury, MA) was thawed on ice overnight in the cold room, and used the next day. The Tufts University Institutional Animal Care and Use Committee approved all animal protocols and procedures (Protocol Number: B2012-62). Cells continually remodel the ECM composition and functionality through the activity of proteolytic enzymes, including neutrophil and macrophage-synthesized elastase, along with the Cu2+ and Zn2+-dependent matrix metalloproteinases (MMPs). pmid:16921413 26. Two room temperature detergent treatments were performed, each lasting 15 minutes; subsequently, the decellularized ECM was washed with five times with TBS (1 minute per wash), immediately collected, and used for enzymatic digestion and peptide identification. Shi L, Ermis R, Garcia A, Telgenhoff D, Aust D. J Burns Wounds 2005;4:e8. While its clinical application as a debriding agent has been associated with improved healing outcomes, questions remain as to whether it actually stimulates tissue repair as an active healing entity or merely prepares the wound bed for healing via the removal of nonviable eschar. Interestingly, Chung, et al [56] revealed that mammalian collagenase 1 (MMP1) must unwind helical structures prior to peptide bond cleavage, as the intact collagen triple helix is too large to fit in the active site cleft of the enzyme. pmid:24291155 5. pmid:14757419 21. The peptides extracted from fibroblastic ECM found at ~65 kDa (Table 1) have their origins in fasciclin-1 (TSN11) and N-terminal domains (TSN12) of TGFβ-induced protein ig-h3 (TGFBI), fibronectin-type III domains of tenascin C (TSN14, 15), and von Willebrand Factor A (vWFA) domains of the type VI collagen α3 chain (TSN16, 17). Based on our results from experiments with purified Clostridial collagenase, we hypothesized that the enzyme combination contained in Santyl® may induce key cellular responses to injury through the production of matrix fragments from specific cleavages made during the debridement process, which stimulates downstream healing activities [25–27]. Ability of chronic wound fluids to degrade peptide growth factors is associated with increased levels of elastase activity and diminished levels of proteinase inhibitors. Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. Endothelial cell-pericyte interactions stimulate basement membrane matrix assembly: influence on vascular tube remodeling, maturation, and stabilization. pmid:12821648 40. Along with this, MMP cleavage liberates ECM-bound angiogenic growth factors, and exposes cryptic bioactive matrix domains that are contained within their 'parent' ECM components, which may lack active healing activities themselves [7,12]. However, the mechanisms by which debridement might promote healing are not fully understood. Immediately prior to use, the enzymes were thawed, diluted to 1000 U/mL in TBS containing 2 mM calcium, and added to the decellularized plates for 24 hours at 37°C. Microsc Microanal 2012;18:68–80. 31. Importantly, mouse wounds treated with TSN18, a novel 'combinatorial' peptide created from fragments of non-helical regions of collagen VI released after digesting fibroblastic ECM with Santyl®, display marked improvements in granulation tissue formation and wound re-epithelialization that go well beyond wounds treated under control conditions and are similar to wounds treated with equivalent doses of Santyl® itself. Additionally, amino acids 17–35 of this domain constitute a calreticulin (CRT)-binding sequence that induces endothelial cell and fibroblast focal adhesion disassembly and migration, as well as resistance to anoikis in vitro, via signals transduced through a CRT/low-density lipoprotein receptor-related protein-1 (LRP1) co-complex [39,40]. Extracellular matrices were prepared as previously described [26] and based on the original methods developed [29]. All surgeries were performed under ketamine and xylazine-induced general anesthesia, and Buprenex was administered subcutaneously for perioperative analgesia. (G). When we apply these peptides to CY-treated, healing-impaired mice, we observe TSN18 stimulates dose-dependent increases in wound re-epithelialization after full-thickness excisional wounding: 1.0 mg/mL TSN18 produces an effect size similar to treatment with 1.0 mg/mL Santyl® collagenase, each yielding a 60% increase in epithelialization over CMC, while treatment with 0.1 mg/mL TSN18 stimulates a >100% increase of epithelial responses over CMC in vivo (Fig 5). Clin Ther 2013;35:1805–20. HBTU activation for automated Fmoc solid-phase peptide synthesis. FASEB J 2008;22:3968–79. 12. Linked to these experimental goals, LC-MS/MS [31] was performed on ~25 kDa and ~65 kDa protein bands excised from SDS gels containing electrophoresed HMVEC or HDF ECM extracts, respectively. Degradation of human collagen isoforms by Clostridium collagenase and the effects of degradation products on cell migration. pmid:15021091 24. N Engl J Med 1999;341:738–46. Young WC, Herman IM. Demidova-Rice TN, Geevarghese A, Herman IM. Along with this, Clostridial collagenase treatment induces cellular responses to injury in vivo, yielding enhanced re-epithelialization and hastened wound closure in Yucatan microswine [25]. pmid:19443898 20. pmid:17476353 16. Despite its reduced ability to proteolyze collagen VI, perhaps due to an inhibitory structure at the C-terminus of the α3 chain [47], the inherent differences between the ways in which the enzymes degrade individual, purified matrix proteins vs. Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, et al. Extracellular matrix signaling in morphogenesis and repair. Mutations in COL6A3 Cause Severe and Mild Phenotypes of Ullrich Congenital Muscular Dystrophy. (E) The lengths of human dermal capillary endothelial sprouts cultured on growth factor-reduced Matrigel in the presence or absence of 100 nM ECM-derived peptides were measured as described in "Methods" at 5 hours post-plating, with physiologic doses of FGF2 (0.6 nM) serving as positive controls. Orr . For peptide identification, soluble and insoluble fractions of HMVEC and HDF matrices digested with Santyl® collagenase were collected into ECM-solubilization buffer containing 20 mM Tris-Cl (pH 8.0), 150 mM NaCl (pH 7.0), 0.1% sodium dodecyl sulfate (SDS), 0.5% sodium deoxycholate, 1% NP-40, supplemented with protease inhibitors (P8340, Sigma-Aldrich). Newman AC, Nakatsu MN, Chou W, Gershon PD, Hughes CC. Subsequently, the chamber slides containing GFR Matrigel mixtures were placed in tissue culture incubators at 37°C for 45–60 minutes to allow for matrix polymerization. Yager D, Chen S, Ward S, Olutoye O, Diegelmann R, Cohen K. Bioactive endothelial and fibroblastic matrix-derived peptides increase human keratinocyte proliferation in vitro.Adult normal human epidermal keratinocytes (NHEK) grown in 48-well plates were treated with peptides at 10 nM every other day for a period of 5 days, and cell counts were performed on Day 5 as described in "Methods." Relative proliferation compared with 50% basal NHEK media (control) is shown as fold of control, calculated by normalizing the mean cell count for each treatment to the mean cell count obtained for negative control, and error bars represent standard deviation. pmid:22710795 46. Euthanasia was performed by lethal CO2 inhalation. Wells were then inoculated with 5X104 HMVEC in DMEM supplemented with 1% BCS +/- FGF2 or the corresponding peptides. Demir E, Sabatelli P, Allamand V, Ferreiro A, Moghadaszadeh B, Makrelouf M, et al. pmid:18661336 55. Fields CG, Lloyd DH, Macdonald RL, Otteson KM, Noble RL. Nam J-OO, Son H-NN, Jun E, Cha K, Lee B-HH, Park R-WW, et al. Acute cutaneous wound healing occurs through a well-orchestrated series of events that culminate in tissue repair and wound closure [1]. Journal of Cellular Physiology 2008;214:828–37. Cha J, Kwak T, Butmarc J, Kim T-AA, Yufit T, Carson P, et al. Drafted the original manuscript: ARS IMH. Full-thickness excisional wounding was performed using sterile 4mm-diameter punch biopsy tools, sterile scissors, and sterile forceps. Falanga V. The authors would like to thank Tufts University Core Facility for peptide synthesis and Dr. Jennifer T. Representative photomicrographs of H&E-stained sections of wounds treated with CMC (A), 0.1 mg/mL TSN6 (B), 1.0 mg/mL TSN6 (C), 0.1 mg/mL TSN18 (D), 1.0 mg/mL TSN18 (E), and Santyl® Collagenase (F) are shown. The funders had no role in study design, data collection or analysis, but were involved in decisions to publish and in editing the manuscript.Competing interests: IMH is inventor of the peptides described in this study. Appl Environ Microb 2015;81:6098–107. Willis CD, Poluzzi C, Mongiat M, Iozzo RV. Several of the matrix-derived peptides increase endothelial cell proliferation by 50–100% over controls, namely the individual endothelial matrix peptides TSN1, 2, and 6, and the combinatorial peptide TSN10, along with the individual fibroblastic matrix peptides TSN12 and 15, and the combinatorial fibroblastic ECM peptides, TSN13 and TSN18 (Fig 1). Matrikines and matricryptins: Implications for cutaneous cancers and skin repair. Ramundo J, Gray M. While these events are likely important for wound-healing angiogenesis and granulation tissue formation, intriguingly, in vivo implantation of sponges containing plasmids encoding this peptide stimulates the production of a dense, collagen-rich capsule that surrounds the implant, mimicking elements of the foreign body response [41]. Curiously, collagen VI is also a major component of white adipose ECM, where proteolytic cleavage releases its bioactive C5 domain, known as endotrophin, which upregulates pro-fibrotic and pro-inflammatory genes in white fat and promotes tumor progression [49,50]. Waycaster C, Milne C. In either case (e.g. native vs. FEBS J 2013;280:2271–84. Over 100 protein fragments were identified in Santyl®-digested matrices; of these, 8 peptides derived from HMVEC matrix and 6 peptides derived from HDF matrix, each containing 12–25 amino acids, were selected and submitted to Tufts University Core Facility (TUCF) for synthesis by FastMoc Chemistry, as previously described [26,30,31]. Dynamic reciprocity in the wound microenvironment. denatured collagen), the distinct cleavage patterns of mammalian and Clostridial collagenases dictate the specific peptides generated. Nagase H, Fushimi K. Blood Cells Mol Dis 2004;32:88–94. Protein bands of interest were excised from the gels, and submitted to Taplin Mass Spectrometry Facility at Harvard Medical School for protein identification. Epithelialization in Wound Healing: A Comprehensive Review. Yates JR, Eng JK, McCormack AL, Schieltz D. Therefore, the removal of nonviable tissue, slough and eschar through debridement is a critical element of preparing a wound bed to heal [20,21]. Primary adult human dermal fibroblasts (HDF, Lonza) were grown in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies, Grand Island, NY) supplemented with 10% FBS (Atlanta Biologicals, Atlanta, GA), 1% antibiotic-antimycotic, 1% L-glutamine, and 10mM HEPES (Life Technologies, Grand Island, NY). PLoS ONE 11(7): e0159598. Since our previous studies indicate that pure Clostridial collagenase significantly promotes cell migration and proliferation in vitro and stimulates wound healing in vivo [25] through liberation of bioactive, matrix-derived peptides [26,27], we aimed to reveal whether Santyl® collagenase, through its proven and selective debridement activity, similarly liberates specific collagen- and collagen-associated peptides that promote cellular responses to injury, including cellular growth and capillary endothelial cell-driven angiogenesis in vitro; and, if so, whether such bioactive peptides could further induce wound-healing angiogenesis and re-epithelialization in murine models of impaired healing [27]. Debridement: controlling the necrotic/cellular burden. Following Santyl® collagenase-digestion of HDF matrices, several unique peptides are liberated, including those derived from TGFβ-induced protein ig-h3 (TGFBI, TSN11, 12), tenascin-C (TSN14, 15), and the α3 chain of collagen VI (TSN16, 17). The morphogenic capacity of the ECM-derived peptides was evaluated as previously described [26,27], with the following modifications. Indeed, the authors further demonstrated that mutating a key catalytic amino acid residue abolishes MMP1 proteolysis while preserving its ability to unwind the collagen triple helix, rendering collagen susceptible to degradation by otherwise non-collagenolytic enzymes [56]. For each treatment condition, relative proliferation was calculated by normalizing the average cell count obtained from the three technical replicates against the average cell count for control treatments. This enzymatically driven ECM turnover is regulated by the tissue inhibitors of metalloproteinases (TIMPs), which bind to and inactivate MMPs [13]. Arteriosclerosis, Thrombosis, and Vascular Biology 1987;7. Though it is unclear whether this latter observation is due to the activity of wholly intact TGFBI per se, it is possible that the mammalian proteases required for ECM remodeling during angiogenesis also cleave TGFBI, liberating morphogenic peptides similar to TSN11/12. At the same time, outcomes from debridement vary across the spectrum of care delivery and the technical means by which debridement is achieved. Collagenase Santyl® Ointment, a sterile enzyme preparation that contains a mixture of collagenases and non-specific proteases produced by Clostridium histolyticum fermentation, is currently the only biologically active enzymatic debridement agent approved by the FDA for clinical use. Download: Fig 5. Based on our previous studies of pure Clostridial collagenase, we now ask whether the mixture of enzymes contained within Santyl® produces matrix-derived peptides that promote cellular injury responses in vitro and stimulate wound closure in vivo. J Dermatol Sci 2008;50:15–23. Intriguingly, while collagenase Santyl® ointment, a sterile preparation containing Clostridial collagenases and other non-specific proteases, is a well-accepted enzymatic debridement agent, its role as an active healing entity has never been established. Syndecan-4 contributes to endothelial tubulogenesis through interactions with two motifs inside the pro-angiogenic N-terminal domain of thrombospondin-1. 58. Cells in the remaining plates were fed with culture media containing 1% BCS for HMVEC, 1% FBS for HDF, or 50% basal keratinocyte growth media (serum-free media with bovine pituitary extract and recombinant human epidermal growth factor) for NHEK. Durham for help with animal experimentation. Statistical analyses of peptide treatments versus negative controls were performed using an unpaired Student's T-test, with p-values < 0.05 considered to be statistically significant. Endothelial cell matrices modulate smooth muscle cell growth, contractile phenotype and sensitivity to heparin. Cell counts were performed on day 5 post-plating. Diversity, Structures, and Collagen-Degrading Mechanisms of Bacterial Collagenolytic Proteases. The requirement for fibroblasts in angiogenesis: fibroblast-derived matrix proteins are essential for endothelial cell lumen formation. Colombatti A, Spessotto P, Doliana R, Mongiat M, Bressan GM, Esposito G. Adv Skin Wound Care 2004;17:66–75; quiz 76–8. The Clinical Relevance of Debridement 1999:17–31. Adult Balb/c mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and housed in the Tufts Division of Laboratory Animal Medicine (DLAM). Adipocyte-derived endotrophin promotes malignant tumor progression. pmid:22384158 28. Bioactive peptides derived from vascular endothelial cell extracellular matrices promote microvascular morphogenesis and wound healing in vitro. After fixation for 24 hours, tissues were embedded in paraffin, cut into 5 μm-thick sections, and stained with H&E or Masson's trichrome. Am J Pathol 2010;177:1710–24. Mol Cancer Res 2012;10:1010–20. At Taplin, the gel bands were degraded using proteomics-grade trypsin, and subjected to liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using Orbitrap mass spectrometers (ThermoFisher Scientific). TGFBIp/betaig-h3 protein: a versatile matrix molecule induced by TGF-beta. Similarly, wet-to-dry dressings that allow mechanical debridement are even less selective and may also remove viable tissue thus creating a larger wound and more pain for patients. The Journal of Cell Biology 2003;161:1179–89. Data are displayed as fold increase in tube length, relative to 1% BCS-treated negative controls, and error bars represent standard deviation. Mean values were compared for each treatment group, and data are displayed as mean fold change relative to negative controls, +/- standard deviation. Wound Repair Regen 2011;19:134–48. J Am Coll Clin Wound Spec 2014;6:14–23. * p < 0.05, ** p < 0.01, compared with CMC-treated controls. Interactions between extracellular matrix and growth factors in wound healing. Further, the ECM is a growth factor repository, capable of binding and sequestering pro-healing molecules such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGFβ), vascular endothelial growth factor (VEGF), and fibroblast growth factor-2 (FGF2), the latter of which possesses bioactivity dependent upon interactions with heparan sulfate proteoglycan (HSPG) [7]. All treatments were administered daily for a period of 5 days, beginning on the first day after injury, and dressings were changed as necessary. A recombinant NH2-terminal heparin-binding domain of the adhesive glycoprotein, thrombospondin-1, promotes endothelial tube formation and cell survival: a possible role for syndecan-4 proteoglycan. those present within a complex, biosynthesized ECM may account for the presence of collagen VI fragments generated from fibroblastic matrices digested with Santyl® collagenase. Cellular and molecular basis of wound healing in diabetes. Matrix-derived signals transduced via integrins and other cell surface receptors regulate platelet activation and clot formation after injury, facilitate monocyte chemotaxis and local macrophage differentiation, and augment phagocytic capacity [3,5]. Circ Res 2007;100:1673–85. pmid:16824016 3. Sweetwyne MT, Pallero MA, Lu A, Van Duyn Graham L, Murphy-Ullrich JE. Wounds were dressed with Tegaderm (3M, St. Paul, MN) immediately after injury. Additionally, matrix-integrin interactions dictate growth and migration of keratinocytes, fibroblasts, and vascular endothelial cells, control morphogenic processes such as endothelial tube formation during angiogenesis, and determine synthesis of additional matrix components [3,6]. Annu Rev Cell Dev Biol 2006;22:287–309. Panuncialman, Falanga. Cell proliferation assays were performed as previously described [26,27], with the following modifications: cells were plated in their normal growth media at a density of 2,000 cells/well in two 48-well plates; the next day (day 1 post-plating), cells were detached from the culture surface of one plate using trypsin, and baseline cell counts were obtained using a Coulter Counter model Z-II (Beckman Coulter, Inc., Fullerton, CA) according to the manufacturer's instructions. Our tests of growth promotion, migration, and endothelial tube formation in vitro bring to light pleiotropic effects of endothelial and fibroblastic ECM peptides released after Santyl® collagenase digestion. A role for collagenase and matrix building growth factors. Accordingly, it remains to be determined whether a group of human ECM peptides similar to the previously identified bovine ECM fragments liberated from digestion with purified Clostridial collagenase also arise as intermediates during the course of digestion with Santyl® collagenase. Overall, the loss of ECM-derived signals and trophic factors stemming from MMP-to-TIMP imbalance drastically alters cell-matrix interactions in chronic wounds and impairs post-injury migration, proliferation, and angiogenesis. Subsequently, CY-treated mice underwent IACUC-approved cranial excisional wounding surgeries as previously described [27], with the following modifications. Endothelial and fibroblastic ECM-derived peptides enhance human dermal fibroblast proliferation in vitro.Dermal fibroblasts isolated from healthy adult human donors grown in 48-well plates were treated with peptides at 100 nM every other day for a period of 5 days, and cell counts were performed on Day 5 as described in "Methods." Relative proliferation compared with 1% FBS (control) is shown as fold of control, calculated by normalizing the mean cell count for each treatment to the mean cell count obtained for negative control, and error bars represent standard deviation. LS and KG are paid employees of Smith & Nephew, PLC. Contributed to manuscript review, editing, and approved the final manuscript draft, which was submitted: ARS TNDR LS VR KVG IMH.1. Singer AJ, Clark RA. J Wound Ostomy Continence Nurs 2009;36:S12–6. CMC alone or CMC containing Santyl® collagenase (1.0 mg/mL) served as negative and positive controls, respectively. The peptides identified in our study are derived from ECM biosynthesized by healthy cells in vitro, which contains molecules in their native states; at the same time, it is notable that the necrotic eschar within non-healing wounds is largely comprised of denatured collagens [51]. Although collagenase Santyl® ointment, which contains a mixture of Clostridial collagenases and other Clostridial proteases, is a well-accepted enzymatic debridement agent, its status as an active healing entity remains unknown. Enzymatic debridement with collagenase Santyl® has also been associated with improved healing dynamics [28], however the molecular mechanisms linked to these clinical outcomes have neither been characterized nor well understood. Contributed on the experimental design, execution, analysis and interpretation: TNDR ARS. Primary adult human dermal microvascular endothelial cells (HMVEC, Lonza Bioscience, Walkersville, MD) were cultured in Endothelial Basal Medium-2 (Lonza) supplemented with 5% fetal bovine serum (FBS) and the growth factors contained within the EGM2-MV kit (Lonza), according to the manufacturer's instructions. Mol Biol Cell 2011;22:3791–800. Enzymatic debridement agents, such as Clostridial collagenase, selectively and painlessly degrade devitalized tissue. pmid:16984454 18. At the same time, our data reveal novel functions of peptides and protein domains previously unknown to participate in wound healing, as this is the first study describing bioactivities of peptides corresponding to amino acids 207–221 (TSN1) and 174–190 (TSN2) of the TSP-1 N-terminal heparin-binding domain. Schultz GS, Davidson JM, Kirsner RS, Bornstein P, Herman IM. Economic and clinical benefit of collagenase ointment compared to a hydrogel dressing for pressure ulcer debridement in a long-term care setting. That some of the endothelial ECM peptides provoke responses in fibroblasts and vice versa may not be so surprising, as the individual contributions of microvascular endothelial and mural cells to their shared basement membrane are known to modulate the behaviors of both cell types during vascular remodeling [58,59]. Optimizing Wound Bed Preparation With Collagenase Enzymatic Debridement. Herman IM. Vitam Horm 2000;59:99–131. Indeed, mutations in the vWFA domains of collagen VI α3 have been connected with congenital myopathies [48], supporting the trophic role of these motifs; at the same time, the bioactivity of TSN18 points to a novel function of the non-helical domains of this protein. From the proteolysed endothelial ECM, we identified peptides (Table 1) that correspond to the N-terminal laminin G-like heparin-binding domain of TSP-1 (TSN1, 2), EGF-like (TSN3), globular C1q (TSN4) and coiled-coil domains (TSN5, 6) of MMRN-1, and heparin- (TSN7) and fibrin-binding regions (TSN8) of fibronectin. Demidova-Rice TN, Wolf L, Deckenback J, Hamblin MR, Herman IM. As collagenase-mediated disruptions in the native triple helical winding structure lead to collagen denaturation without impacting the primary amino acid sequence, it is therefore likely that the peptides released from native collagen are highly similar to those liberated from denatured collagen by the activity of either enzyme. Brown BN, Badylak SF. pmid:18078741 45. The ischemia resulting from angiogenic impairment can lead to wound necrosis [18,19]. Previous studies have demonstrated Clostridial collagenase degrades purified, full-length collagen VI, albeit to a lesser degree than other purified, full-length collagen molecules, including collagens I, III, IV and V [47]. McCarty SM, Percival SL. Removing necrotic slough and eschar through surgical or mechanical methods is less specific and may be painful for patients. Provided reagents: LS. In addition, and as we have previously reported [26,27], we have recombined several domains or fragments from a few of the peptides identified, creating 'combinatorial' peptides to explore whether such recombined peptides that possess multiple, key signaling domains might be more efficacious in promoting wound healing responses in vitro or in vivo. The enhancements in granulation tissue deposition and wound re-epithelialization may occur through the release of several bioactive ECM peptides, which stimulate human dermal endothelial, keratinocyte, and fibroblast proliferation, as well as microvascular morphogenesis in vitro. pmid:9893278 7. J Dermatol Sci 2005;40:11–20. pmid:12730128 14. pmid:18079100 11. Indeed, the distinctions between ECM peptides liberated through the activity of specific proteolytic enzymes cannot be understated, as evidenced from our past studies. Relative endothelial tube formation in each experiment was calculated by normalizing the average sprout length measured for each condition to the average sprout length for control treatments. Endotrophin triggers adipose tissue fibrosis and metabolic dysfunction. Clostridial collagenase releases bioactive fragments from extracellular matrix molecules. ARS, TDR and VR have declared that no competing interests exist. pmid:17585075 6. pmid:21362080 4. Here, we identify novel collagen fragments, along with collagen-associated peptides derived from thrombospondin-1, multimerin-1, fibronectin, TGFβ-induced protein ig-h3 and tenascin-C, generated from Santyl® collagenase-digested human dermal capillary endothelial and fibroblastic matrices, which increase cell proliferation and angiogenic remodeling in vitro by 50–100% over controls. The N-terminus of thrombospondin: the domain stands apart. Enzymatic ECM digestion was performed as previously described [26] with the following modifications: lyophilized Santyl® collagenase (the drug substance for Santyl®) was obtained from Smith & Nephew PLC (Fort Worth, TX), reconstituted in TBS at 1.0 mg/mL, and stored at -20°C before use for no longer than 1 week. Tallis A, Motley TA, Wunderlich RP, Dickerson JE, Waycaster C, Slade HB. Riley KN, Herman IM. Expert Rev Mol Med 2011;13:e23. J Cell Sci 1985;73:19–32. pmid:15094124 33. 22. pmid:15993569 13. Conceived and designed the experiments: IMH TNDR ARS VR. This devitalized tissue, largely comprised of denatured collagen, may exacerbate inflammation, increase susceptibility to infection, and presents an impediment to reparative processes. Briefly, animals were treated with two doses of pharmaceutical grade cyclophosphamide (CY, Sigma-Aldrich) dissolved in sterile saline, administered via intramuscular injection into the caudal thigh at 4 days (150 mg/kg) and 1 day (100 mg/kg) prior to injury. pmid:25866979 23. Further, it may be of vital importance to combine those peptides previously identified as bioactive wound healing entities [26,27] and those discovered, herein, to learn whether their combined application further enhances wound-healing angiogenesis and re-epithelialization compared to single agent administration. Yoshida N, Lauer-Fields JL, Fields GB. J Wound Ostomy Continence Nurs 2009;36:S4–11. These results not only confirm and extend our earlier studies using purified collagenase- and matrix-derived peptides to stimulate healing in vitro and in vivo, but these Santyl®-generated, matrix-derived peptides may also represent exciting new opportunities for creating advanced wound healing therapies that are enabled by enzymatic debridement and potentially go beyond debridement. Thus, we created combinatorial peptides comprised of individual domains contained within the HMVEC (TSN9, 10) and HDF (TSN13) matrix-derived peptides, or which contain fragments of additional matrix protein domains identified through LC-MS/MS (TSN18, 19). Indeed, HMVEC- or HDF-derived ECM digested with Santyl® collagenase yield several distinct protein fragments. Primary adult human epidermal keratinocytes (NHEK, Lonza) were cultured as previously described [27]. Tran KT, Lamb P, Deng J-SS. Wound Repair Regen 2009;17:153–62. The science of wound bed preparation 2009. Collagenase-liberated protein fragments and ECM-derived peptides include the α3 chain of type VI collagen, as well as peptides derived from thrombospondin-1 (TSP-1), multimerin-1 (MMRN-1), and fibronectin, to name several. Our earlier and groundbreaking pre-clinical studies indicate that purified Clostridial collagenase stimulates the cellular responses to injury in vitro and promotes wound closure in vivo [24,25]. Human platelet-rich plasma- and extracellular matrix-derived peptides promote impaired cutaneous wound healing in vivo. This does not alter the authors' adherence to all PLOS ONE policies on sharing data and materials. Circ Res 2003;92:827–39. Visse R, Nagase H. In addition, the individual fibroblastic ECM peptides TSN12, 15, 16, and 17 significantly stimulate endothelial morphogenesis in vitro to a similar extent as FGF2, however the increase in tube formation observed following treatment with the combinatorial fibroblastic ECM peptides TSN13, 18 and 19 is not statistically significant. Thrombospondin 1 binding to calreticulin-LRP1 signals resistance to anoikis. Indeed, the TSN11 peptide derived from the TGFBI fasciclin-1 (FAS1) domain stimulates endothelial migration and morphogenesis in vitro, along with fibroblast proliferation, yet the full FAS1 domain has known anti-migratory and anti-angiogenic properties, mediated through disruptions in endothelial integrin αVβ3 and VEGF signaling [45]. Do Outeiro-Bernstein M, Nunes S, Andrade A, Alves T, Legrand C, Morandi V. The endothelial ECM-derived peptides TSN2, 3, 4 and 6 also significantly elevate keratinocyte proliferation by 40–75%, as does the combinatorial peptide TSN10; while several fibroblastic ECM peptides increase NHEK growth by 20–40% over controls, these changes are not statistically significant (Fig 2). Together, these data suggest that the pro-healing activities of Clostridial collagenase are mediated by the liberation of bioactive matrix fragments that function to promote the migratory, proliferative, and angiogenesis-inducing activities required for wound healing. For example, when keratinocytes are plated upon a clostripain-digested endothelial ECM cell growth is not stimulated; however, when purified Clostridial collagenase is then added to the growth media, these very same cells are markedly stimulated to proliferate [25]. Download: Fig 2. pmid:21865599 47. pmid:17659994 44. Peptide treatments were administered in basal media on day 1 and day 3 post-plating, at a concentration of 100 nM. Adv Wound Care (New Rochelle) 2013;2:438–47. Stimulation of human keratinocyte migration and proliferation in vitro: insights into the cellular responses to injury and wound healing. Wrote the paper: ARS IMH. Int J Low Extrem Wounds 2009;8:95–104. Van Zoelen EJ, Stortelers C, Lenferink AE, Van de Poll ML. In the present study, we identify several collagen- and collagen-associated peptides that are liberated due to specific cleavages made when endothelial or fibroblastic ECM are treated with Santyl® collagenase in vitro. Briefly, after euthanasia by CO2 inhalation, cranial wounds, together with underlying fascia and connective tissue, and approximately 5 mm of intact dermal and epidermal tissues surrounding the wounds were excised. Ruggeri ZM, Mendolicchio GL. * p < 0.05, ** p < 0.01, *** p < 0.001, compared with negative control (unpaired Student's T-test). Zhang Y-Z, Ran L-Y, Li C-Y, Chen X-L. Animal tissues were collected at 5 days post-injury as previously described [27]. Herman Castellot. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.Data Availability: All relevant data are contained within the paper.Funding: This work was supported by a sponsored research agreement between Tufts University (IMH) and Smith & Nephew, PLC (. Extracellular matrix modulation of endothelial cell shape and motility following injury in vitro. Cardiovasc Res 2008;78:203–12. Int J Biochem Cell Biol 2004;36:1090–101. Mean proliferation, standard deviation, and statistical significance were calculated from the normalized values derived from each independent experiment. Indeed, our previous studies identified fragments of fibrillin-1, tenascin X, the α1 chain of type I collagen, and the α3 chain of type IV collagen derived from bovine retinal microvascular endothelial ECM digested with pure Clostridial collagenase [26], which we did not detect in our present investigations of human dermal microvascular ECM peptides. Proteases and Delayed Wound Healing. A total of three experiments were performed, and each independent experiment contained three technical replicates for each condition assayed. Thus, while we cannot completely rule out the differences in species or tissue origin, the array of peptides previously identified from bovine endothelial ECM may be specifically linked to the activity of Clostridial collagenase, alone, compared to peptides released from human endothelial ECM as a result of the combined enzymatic activities contained within preparations of Santyl®. Quantitative evaluation of peptide-dependent wound re-epithelialization.Percent wound closure attributable to re-epithelialization was measured from merged photomicrographs of hematoxylin and eosin or trichrome-stained tissue sections using NIH ImageJ as described in "Methods." (A-F). Quantitative analysis of peptide-induced dermal microvascular remodeling in vitro.(A-D) Representative photomicrographs of human dermal capillary endothelial cell morphogenesis following treatment with 1% BCS (A), 100 nM TSN1 (B), 100 nM TSN 8 (C), and 100 nM TSN 10 (D). Elucidating the function of non catalytic domains of collagenases and aggrecanases. Anal Chem 1995;67:1426–36. Our results support several previous studies revealing collagenase stimulation of wound healing [24,25,34], which was later attributed to the proteolytic cleavage of ECM with bacterial collagenase and the liberation of bioactive wound healing peptides derived from collagenous and collagen-associated ECM-derived protein fragments [26,27,35]. The wounds and adjacent uninjured tissues were then bisected; one half was placed in 10% phosphate buffered formalin (ThermoFisher Scientific) and reserved for staining with hematoxylin and eosin (H&E) and Masson's trichrome, and the other half was embedded in Tissue-Tek OCT compound (VWR, Radnor, PA), frozen on dry ice, and reserved for immunohistochemistry. J Cell Biochem Suppl 1998;30–31:250–63. Lelièvre SA, Bissell MJ. pmid:7741214 32. When we treat fibroblasts with TSN1 and 6, proliferation significantly increases by 50–100% over controls; although we observe similar results following treatment with TSN2 and the combinatorial peptide TSN10, these changes are not statistically significant (Fig 3). In vitro assays of peptide bioactivity reveal the naturally occurring endothelial ECM peptide, TSN6, and the combinatorial fibroblastic ECM peptide, TSN18 have the greatest stimulatory activity over all parameters and cell types assayed. J Clin Invest 2012;122:4243–56. To determine the identity of the ECM-derived molecular species released during Santyl® collagenase-mediated ECM digestion, liquid chromatography-tandem mass spectroscopy was performed on select SDS polyacrylamide gel-electrophoresed protein bands of interest and present in the collagenase-digested but not control treated ECM preparations. The non-healing leg ulcer: peripheral vascular disease, chronic venous insufficiency, and ischemic vasculitis. Using an established model of impaired healing, we further demonstrate a specific dose of collagenase from Santyl® ointment, as well as the newly-identified and chemically-synthesized ECM-derived peptides significantly increase wound re-epithelialization by 60–100% over saline-treated controls. pmid:10714238 34. pmid:22566882 43. Haemostasis 1990;20 Suppl 1:166–77. Elzie CA, Murphy-Ullrich JE. Nature Structural & Molecular Biology 2011;18:1109–14. Whereas wounds treated with CMC alone display ~30% closure, administration of 0.1 mg/mL TSN18 results in ~70% closure, and treatment with 1.0 mg/mL TSN18 yields ~50% closure, similar to the effects of Santyl® collagenase. Sharp debridement is a rapid method of clearing tissue, but it is does not selectively remove necrotic material, and results in perioperative site pain. Performed the experiments: ARS TNDR. While 0.1 mg/mL TSN6 induces ~36% wound closure, this change is not significantly different from CMC-treated wounds. Regulation of vascular smooth muscle cell growth by endothelial- synthesized extracellular matrices. In addition to its debriding activities, purified Clostridial collagenase stimulates cellular responses to injury. Shi L, Carson D. These disparate findings suggest unique bioactivies of FAS1 peptides versus the intact domain. In addition to its debriding activities, highly-purified Clostridial collagenase actively promotes healing, and our past studies reveal that extracellular matrices digested with this enzyme yield peptides that activate cellular migratory, proliferative and angiogenic responses to injury in vitro, and promote wound closure in vivo. Che John Connon, University of Reading, UNITED KINGDOMReceived: April 19, 2016; Accepted: July 4, 2016; Published: July 26, 2016Copyright: © 2016 Sheets et al. Mean percent wound closure is displayed, and error bars represent standard deviation

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