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Type VI collagen regulates dermal matrix assembly and fibroblast motility

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ABSTRACT

Type VI collagen is a non-fibrillar collagen expressed in many connective tissues and implicated in extracellular matrix (ECM) organisation. We hypothesized that type VI collagen regulates matrix assembly and cell function within the dermis of the skin. In the present study we examined the expression pattern of type VI collagen in normal and wounded skin and investigated its specific function in new matrix deposition by human dermal fibroblasts. Type VI collagen was expressed throughout the dermis of intact human skin, at the expanding margins of human keloid samples, and in the granulation tissue of newly deposited ECM in a mouse model of wound healing. Generation of cell derived matrices (CDMs) by human dermal fibroblasts with stable knockdown of *COL6A1* revealed that type VI collagen deficient matrices were significantly thinner and contained more aligned, thicker, and widely spaced fibres than CDMs produced by normal fibroblasts. In addition, there was significantly less total collagen and sulfated proteoglycans present in the type VI collagen depleted matrices. Normal fibroblasts cultured on de-cellularised CDMs lacking type VI collagen displayed increased cell spreading, migration speed, and persistence. Taken together, these findings indicate that type VI collagen is a key regulator of dermal matrix assembly, composition, and fibroblast behaviour and may play an important role in wound healing and tissue regeneration.

INTRODUCTION:

Cutaneous wound healing is a complex and dynamic set of processes that begins following injury and is required to re-establish skin integrity and function. Three overlapping phases of wound healing have been described: inflammation, tissue formation and tissue remodelling. Extracellular matrix (ECM) synthesis, assembly and organisation occur during the latter two phases and are essential for restoring tissue strength and structure (Singer and Clark, 1999). Dermal fibroblasts are the main cell type responsible for ECM synthesis, and dysfunctional matrix deposition has been implicated in both chronic, non-healing wounds and scar formation (Caskey *et al.*, 2014; Herrick *et al.*, 1992; Xue and Jackson, 2013). Deciphering the function of specific regulatory molecules involved in these processes could offer new insights into impaired wound healing and identify therapeutic targets for wound repair and tissue regeneration.

Type VI collagen is a non-fibrillar collagen that is expressed in many connective tissues and implicated in matrix organisation. The monomeric unit of type VI collagen is a heterotrimer comprised of three α chains: α1(VI), α2(VI) and α3(VI). The biosynthesis of type VI collagen entails intracellular assembly of the three chains into monomers, dimers and tetramers, followed by secretion and extracellular formation of beaded microfilaments, which are next deposited in the extracellular matrix (ECM) (Chu *et al.*, 1988; von der Mark *et al.*, 1984). Recently, three new chains that closely resemble α3(VI) were identified: α4(VI), α5(VI) and α6(VI) (Fitzgerald *et al.*, 2008; Gara *et al.*, 2008). Unlike α3(VI) however, their distribution is limited to specific tissues such as skeletal muscle, myocardium and reproductive organs (Fitzgerald *et al.*, 2013; Gara *et al.*, 2011; Sabatelli *et al.*, 2011).

Type VI collagen interacts with a multitude of key ECM components, including fibrillar collagens type I (Bonaldo *et al.*, 1990) and II (Bidanset *et al.*, 1992), basement membrane type IV collagen (Kuo *et al.*, 1997), fibronectin (Fitch *et al.*, 1991), glycosaminoglycans hyaluronan and heparin (Specks *et al.*, 1992) and proteoglycans perlecan (Tillet *et al.*, 1994), biglycan and decorin (Wiberg *et al.*, 2001). Within connective tissue ECM, type VI collagen forms a highly branched filamentous meshwork which encircles the fibres of principal fibrillar collagens type I, II and III. In addition, in part via interactions with type IV collagen in basement membranes, it anchors blood vessels, nerves and mesenchymal cells (Keene *et al.*, 1988; Kuo *et al.*, 1997). It also serves as a repository for platelet-derived growth factor (Somasundaram and Schuppan, 1996), keratinocyte growth factor (Ruehl *et al.*, 2002), matrix metalloproteinases -1, -2, -3, -8 and -9 (Freise *et al.*, 2009), interleukin 2 (Somasundaram *et al.*, 2000) and cytokine oncostatin M (Somasundaram *et al.*, 2002), thus mediating their activity and availability.

Mutations in any of the three genes that encode the type VI collagen chains (*COL6A1*, *COL6A2* and *COL6A3*) cause disorders affecting muscle and connective tissue, with clinical features that include muscular weakness and joint contractures and laxity. The two most significant diseases associated with type VI collagen are Ullrich congenital muscular dystrophy (UCMD) and Bethlem myopathy (BM) (Bushby *et al.*, 2014). Notably, abnormal skin findings have been reported in many BM and UCMD patients, such as follicular hyperkeratosis, hypertrophic and keloid scarring, dry skin and striae rubra (Briñas *et al.*, 2010; Collins *et al.*, 2012; Jimenez-Mallebrera *et al.*, 2006; Saroja *et al.*, 2013). Type VI collagen is expressed throughout the dermis of the skin and regulates hair follicle cycling (Chen *et al.*, 2015), but its complete function within the tissue has yet to be determined.

Based on the interactions with other ECM molecules, we hypothesised that type VI collagen directs matrix assembly and influences dermal cell behaviour. The present study therefore aimed to determine the function of type VI collagen within the dermis by analysing cell-derived matrices (CDMs) generated by normal and *COL6A1* deficient human dermal fibroblasts. Our results indicate that type VI collagen regulates both the organisation and composition of *de novo* ECM. Importantly, these changes in the ECM have a significant impact on fibroblast morphology and migration. Together, our findings provide insight into the biological function of type VI collagen within the skin and suggest a key role in wound healing and tissue repair.

RESULTS:

Type VI collagen expression in normal, keloid scars and acute wound healing

Immunofluorescence detection of type VI collagen in frozen sections of human neonatal foreskin samples revealed that type VI collagen was widely distributed throughout the papillary dermis, reticular dermis and hypodermis with a higher expression in the papillary dermis (Figure 1a). A similar expression pattern was observed in adult skin samples with type VI collagen present throughout the dermis, most prominently in the papillary dermis and around the vasculature (Figure 1b). Keloid scarring is a pathological wound healing condition that is characterised by excessive matrix deposition and is thought to advance via a tongue-like expanding edge underneath the healthy epidermis and papillary dermis (Bran *et al.*, 2009). Consistent with a role in ECM assembly, type VI collagen was highly expressed at the edges of keloid scars (Figure 1c). This pattern was observed for all six patient samples examined, which included different ethnicities and scar locations (Supplementary Figure S1 and Table S1), and was distinct from the

pattern of type I collagen and fibronectin, which were observed more uniformly throughout the papillary and reticular dermis (Figure 1d,e). Type III collagen, which also has a role in matrix assembly (Brisson *et al.*, 2015), localised to the papillary dermis and leading edge of keloids, similar to type VI collagen (Figure 1f).

To determine the spatial and temporal expression pattern of type VI collagen during normal wound healing, full-thickness 5 mm biopsy wounds were made on the back skin of C57BL/6 mice. Immunofluorescence staining revealed that type VI collagen was most prominently expressed around the wound edges (white arrows) on days 1 and 3, but not within the wound (Figure 1g,h). On day 7, however, type VI collagen notably increased throughout the granulation tissue within the re-epithelialised wound (Figure 1i). These results are consistent with previous findings (Chen *et al.*, 2015) and suggest an involvement of type VI collagen in cutaneous wound repair, as it is deposited in the early stages of new tissue formation within the dermis.

Generation of COL6A1 knockdown dermal fibroblast cell lines and cell derived matrices

To investigate how type VI collagen regulates matrix assembly by human dermal fibroblasts, we developed an in vitro model of *de novo* matrix synthesis using the neonatal foreskin fibroblast cell line HCA2, immortalised with human telomerase reverse transcriptase (hTeRT) (Stephens *et al.*, 2004). Quantitative RT-PCR (qPCR) analysis confirmed that these cells retain 40% *COL6A1* expression compared to primary fibroblasts, while primary keratinocytes express negligible levels (Figure 2a). Lentiviral transduction of three different shRNA constructs and a non-targeting control (NTC) was utilised to develop fibroblast lines with stable knockdown of *COL6A1*. Western blot analysis demonstrated nearly complete knockdown of type VI collagen

for shRNA1 treatment, partial knockdown for shRNA2 and no knockdown for shRNA3 (Figure 2b, bottom). This result was corroborated at the mRNA level with qPCR analysis, which showed 95% knockdown for shRNA1 and 60% knockdown for shRNA2 (Figure 2b, top). Only shRNA3 adversely affected metabolic activity (Supplementary Figure S2). Based on these findings, shRNA1 (sh1) and shRNA2 (sh2) were selected for further experiments.

These cell lines were subsequently employed to produce cell-derived matrices (CDMs), as outlined in Figure 2c. Allowing cells in long term culture to create their own ECM yields a naturally synthesised three dimensional and organised ECM structure that can function as a tissue engineering scaffold with *in vivo* like features (Cukierman *et al.*, 2001; Ishikawa *et al.*, 1997). Immunofluorescence staining of the de-cellularised matrices confirmed the low quantity of type VI collagen in the sh1 CDM (Figure 2d bottom) as opposed to a type VI collagen rich matrix generated from NTC cells (Figure 2d top). No compensatory effects of *COL6A1* knockdown on gene expression of other type VI collagen chains (*COL6A2*, *COL6A3*, *COL6A5* and *COL6A6),* other collagen types, or fibronectin were detected (Supplementary Figure S3). At the protein level, depletion of the α1(VI) chain prevented release of α2(VI) into the media, and both the α2(VI) and α3(VI) chains were retained intracellularly (Supplementary Figure S4a). We confirmed that this intracellular retention did not activate an unfolded protein response (UPR), as there were no significant changes in mRNA levels of UPR genes following *COL6A1* knockdown (Supplementary Figure S4b) (Pan *et al.*, 2013; Hicks *et al.*, 2014).

Type VI collagen regulation of ECM structure and composition

To determine the role of type VI collagen in *de novo* ECM assembly, cell derived matrices were produced from sh1, sh2 and NTC HCA2 cell lines. The *COL6A1* knockdown cells (sh1 and sh2) generated matrices with more aligned fibronectin fibres as evidenced by both the representative immunofluorescence images (Figure 3a) and the corresponding directionality analysis (Figure 3b), which indicates the frequency of alignment angles for the fibres. The level of alignment was quantified by calculating the standard deviation of the Gaussian distributions of all the directionality histograms, and the fibronectin fibres of sh1 and sh2 derived matrices had significantly narrower distributions of fibre orientations than the control matrices (Figure 3c, second graph). The thickness of the entire matrix was also found to be significantly greater for the control compared to the sh1 and sh2 matrices (Figure 3c, first graph). Finally, sh1 cells generated thicker fibres and had greater interfibrillar spacing compared to control and sh2 (Figure 3c, third and fourth graphs). The intermediate changes in total matrix thickness and fibre alignment with the partial knockdown (sh2) indicate a dose-dependent and specific effect of *COL6A1* knockdown on ECM organisation. Sh1 and NTC cell lines were used in all further experiments.

To specify when the effects of type VI collagen depletion arise, fibronectin organisation was examined over a 10 day time-course. On day 3 the matrices appeared similar, but by day 5 the differences in alignment were noticeable and intensified for subsequent time points (Figure 3d). The above findings were validated with scanning electron microscopy (SEM) images of the CDMs on days 5 and 10. The control matrices were composed of a dense network of intertwined, mostly randomly oriented fibres, while sh1 matrices were sparser, with thicker, highly aligned

fibres (Figure 3e). This distinctive alignment of fibronectin fibres was also exhibited by matrices obtained by primary fibroblasts with *COL6A1* knockdown, confirming that the HCA2 model accurately reflects the behaviour of primary fibroblasts (Supplementary Figure S5). We therefore conclude that type VI collagen is required for fibroblasts to assemble a dense ECM with small randomly aligned fibres.

To gain further understanding of how type VI collagen affects matrix composition, the CDMs were stained with antibodies against a panel ECM proteins (Figure 4a). Type I collagen was more abundant in the control matrices, which also retained their random alignment and thinner fibre network. Tenascin C and vitronectin were present at similar levels in both CDMs and appeared throughout the fibrillar network. Versican appeared in "pockets" within the CDM with a higher level in the NTC matrices. Finally, similar levels of decorin were observed on both CDMs. Biochemical assays quantitatively confirmed the immunofluorescence findings. Both pepsin-acid soluble, undenatured collagen measured with the Sircol™ assay and total collagen content measured with the Hydroxyproline assay were significantly higher for control CDMs compared to sh1 matrices (Figure 4b and c). Control CDMs contained higher sulfated glycosaminoglycan (sGAG) content than sh1, as quantified with the DMMB assay (Figure 4d). The different matrices contained the same number of cells; approximately 180,000 cells (Figure 4e). Collectively, these findings demonstrate that type VI collagen regulates the composition of ECM deposited by dermal fibroblasts.

Effect of type VI collagen on human dermal fibroblast spreading and motility

We next interrogated how loss of type VI collagen in the ECM influenced cell behaviour. Primary foreskin dermal fibroblasts at passage five were seeded onto de-cellularised control and knockdown CDMs, and cell spreading, morphology, proliferation, differentiation, alignment and migration were assessed. Two dimensional fibronectin (Fn) coated glass coverslips were used as an additional comparison. Following twenty-four hours on the different substrates both CDMs promoted similar levels of cell attachment, which was significantly higher than 2D Fn (Figure 5a,b, left). Fibroblasts on the CDMs adopted an elongated, spindle shape (Cukierman *et al.*, 2001) as opposed to more round and spread morphologies on the Fn control. The cell area was largest on the Fn control, 2704 ± 112.5 μm², followed by sh1 CDM, 2327 ± 115.7 μm², and NTC CDM 1901 ± 81.63 μm² (Figure 5b, middle). Interestingly, cells on the knock-down CDMs appeared to have more projections (Figure 5a). Finally, cell aspect ratio was significantly higher on both CMDs, than the Fn control (Figure 5b, right).

Type VI collagen has been associated with myofibroblast differentiation (Naugle *et al.*, 2006) and is expressed by adipocytes (Pasarica *et al.*, 2009). After 7 days of culture, almost all of the fibroblasts were α-smooth muscle actin (aSMA) positive on the CDMs and less so on the Fn control (Figure 5c and 5d, left). The percentage of Ki67 positive cells was also higher on the CDMs than the Fn control (Figure 5c and 5d right). Oil red O staining of the cultured cells revealed complete absence of adipogenic differentiation for all three conditions (Figure 5e).

We then co-stained for phalloidin and fibronectin to visualise the cells in relation to the CDMs. Cells on the type VI collagen depleted CDMs were oriented parallel with the Fn fibres, in

contrast with a more random orientation for cells on the control CDMs (Figure 6a). We also explored the role of type VI collagen in fibroblast migration by time-lapse microscopy on the three different substrates. The cells displayed distinct migratory behaviours: they travelled the most distance on the sh1 CDMs compared to the NTC and Fn control, as illustrated by the representative cell track plots (Figure 6b) and the migration movies (Supplementary Movie M1). Cells on the sh1 CDMs migrated with the greatest speed, followed by cells on the control CDMs and then 2D Fn (Figure 6c, left). Cells on sh1 CDMs also exhibited slightly higher persistence compared to NTC CDMs and Fn control (Figure 6c, right). Taken together, these results indicate that type VI collagen influences fibroblast spreading, alignment and motility but has no effect on proliferation or differentiation.

The effects of type VI collagen depletion on cell migration could also be observed between NTC CDMs from day 7 and sh1 CDMs from day 10, at which point the thickness of both matrices is approximately 8 μm, and cells migrated similarly on 2D surfaces coated with purified type VI collagen and fibronectin compared to fibronectin alone (Supplementary Figure S6). Consistent with previous reports in which topographical cues from the ECM direct cell migration (Kutys *et al.*, 2013), we also find that dermal fibroblasts migrate along fibronectin coated aligned polystyrene fibres generated by electrospinning (Supplementary Movie S2). These results therefore suggest that type VI collagen-dependent changes in matrix structure, rather than composition, regulate fibroblast motility.

DISCUSSION:

The overall aim of this study was to investigate the role of type VI collagen in the assembly of ECM by human dermal fibroblasts and evaluate the subsequent impact on cell behaviour. By characterising CDMs generated by *COL6A1* knockdown and control fibroblasts we demonstrate that type VI collagen mediates ECM architecture and composition and affects fibroblast morphology and migration. In conjunction with the presence of type VI collagen throughout the dermis of intact skin and within the newly formed granulation tissue of acute wounds, these results suggest that it may play an important role in wound repair. Thus, our findings provide insight into the function of type VI collagen within the skin.

In agreement with our studies, Sabatelli *et al.* observed abnormal fibronectin deposition in the ECM from fibroblasts of a Bethlem myopathy patient and also from *Col6a1* null mice (Sabatelli *et al.*, 2001). Type VI collagen has also been shown to modulate type I collagen fibrillogenesis (Minamitani *et al.*, 2004), and type I collagen fibrils appear irregular in UCMD patients' skin (Kirschner *et al.*, 2005). Consistent with our results in the mouse, type VI collagen is deposited during the early stages of wound healing in human skin, and its synthesis increases in parallel with type I collagen, reaching a peak approximately 8 days post-wounding (Betz *et al.*, 1993; Oono *et al.*, 1993). Although Lettmann and colleagues recently described no effects of type VI collagen deficiency on overall wound closure time, both the tensile strength and collagen fibril architecture were disrupted in the skin of *Col6a1* null mice (Lettmann *et al.*, 2014).

Interestingly, we find that type VI collagen is highly expressed at the expanding margins of keloid scars, not only for Afro-American ear-lobe samples (Peltonen *et al.*, 1991), but also for additional ethnic groups and anatomical sites including abdomen and occipital scalp. Given its role in matrix assembly, type VI collagen may therefore contribute to excess matrix deposition and the pathogenesis of keloid scar formation.

The most striking effect of loss of type VI collagen in the present study was on fibroblast motility. Cells migrated with increased speed and persistence on the highly aligned CDMs lacking type VI collagen. Topography of the fibrillar network likely directs migration; a more aligned matrix provides guidance cues for rapid and directionally persistent cell movement (Kutys *et al.*, 2013). Furthermore, the increased spreading of fibroblasts on the knockdown CDMs could potentially be attributed to the increased interfibrillar spaces. Significantly, these results demonstrate how type VI collagen dependent changes in the ECM directly impact dermal cell behaviour.

While it is clear that type VI collagen plays an key role in regulating dermal tissue architecture and fibroblast function, several important questions remain. For example, it is unclear which specific subdomains within the protein interact with other ECM components to control fibrillogenesis. We speculate that as a microfibrillar collagen that physically interacts with fibrillar ECM components (e.g. fibronectin and collagen I), type VI collagen alters fibrillogenesis by displacing other ECM components, leading to fibril branching and a more randomly oriented matrix. In addition to understanding the molecular mechanisms by which type VI collagen controls matrix assembly, it will also be interesting in future studies to explore

its role in ageing and other skin pathologies, such as chronic wounds. Finally, type VI collagen could be a potential therapeutic target for tissue repair. Delivery of exogenous type VI collagen may be advantageous for stimulating matrix deposition, while inhibiting its expression may be desirable for reducing fibrosis and scar formation. Thus, tuning the levels of type VI collagen within the tissue could represent an approach to modulating tissue regeneration.

MATERIALS AND METHODS:

Human tissue samples

Skin samples were obtained from keloid patients and healthy volunteers from the plastic surgery department at Barts Health NHS Trust. Foreskin samples were obtained from neonatal circumcision procedures. All subjects gave written, informed consent and the study was conducted under local ethical committee approval (East London Research Ethics Committee, study no 2011-000626-29) and according to the Declaration of Helsinki. Details of patient skin are listed in Supplementary Information Table S1.

Cell culture

Primary dermal fibroblasts and HCA2 cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing GlutaMAX™ (Gibco, Invitrogen, Paisley, UK), 10% fetal bovine serum (FBS) (labtech.com, Uckfield, UK) and 1% penicillin and streptomycin (Gibco, Invitrogen) at 37°C in a humidified atmosphere of 5% CO_2/95% air. The culture medium was changed every two to three days and the cells were passaged at 70 to 80% confluency.

CDMs were generated as previously described with minor modifications (Beacham *et al.*, 2007). Briefly, sterile 13 mm glass coverslips were incubated with 0.2% sterile gelatin for 60 min at 37°C (gelatin type B, Sigma). They were washed 3 times with PBS, cross-linked with 1% sterile glutaraldehyde for 30 min at room temperature (RT) and again washed 3 times with PBS. Cross-linking was quenched with 1M sterile glycine in PBS for 20 min at RT, followed by 3 times PBS washing and incubation in growth medium (DMEM, 10% FBS, 1% P/S) for 30 min at 37°C and finally 3 times PBS washing. The coverslips were either used immediately or stored at 4°C for a maximum of 4 weeks. Dermal fibroblasts were plated at a density of 60,000 cells per well and cultured overnight in 37°C, 5% CO_2 to achieve full confluency. Medium supplemented with 50 µg/ml ascorbic acid was added and subsequently changed every two days. Cells were cultured for 10 days.

To denude cells, medium was aspirated and cells were washed once with PBS. Pre-warmed extraction buffer was then added (10mM NH_4OH, 0.25% Triton X-100 in PBS) and left for 4 min to allow cell lysis. Half of the buffer was carefully removed and PBS was added (Gibco, Invitrogen). The same step was repeated until no intact cells were visible. The DNA residue was digested with 10 µg/ml DNase I (Roche) in PBS for 30 min at 37°C, 5% CO_2, followed by two washes with PBS. Matrices were stored at 4°C in PBS with pen/strep for up to 4 weeks.

See Supplementary Information for additional materials and methods.

CONFLICT OF INTEREST:

The authors state no conflict of interest

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FIGURE CAPTIONS

Figure 1: Type VI collagen expression in normal and wounded skin. (a) Representative image of type VI collagen localisation (red) in frozen sections of neonatal foreskin. (b-c) Representative images of type VI collagen localisation (red) in paraffin embedded sections of (b) adult abdominal skin or (c) abdominal keloid scars. Samples were co-stained for keratin 14 or CD31 (green) to demarcate the epidermis or blood vessels, respectively. Keloid sections were also stained for type I collagen (d) type III collagen (e), and fibronectin (f). Arrows indicate the scar edge. (g-i) Immunofluorescence images of type VI collagen (red) and keratin 14 (green) in frozen sections of wounded mouse skin at days (g) 1, (h) 3, and (i) 7 post-wounding. Excisional wounds were made on the back skin of 6-8 week old, male C57Bl/6 mice using a 5 mm biopsy punch. Arrows denote wound margins. F=fibrin clot, GT=granulation tissue, NE= neo-epidermis. Scale bars: 100 μm. Insets are 2X zoom.

Figure 2: shRNA mediated knockdown of *COL6A1* in dermal fibroblasts. (a) Quantification of *COL6A1* expression in cultured primary human dermal fibroblasts, HCA2 fibroblasts, and primary human keratinocytes by qRT-PCR. Expression was normalised to *GAPDH* and expressed relative to primary fibroblasts. (b) QRT-PCR analysis of *COL6A1* expression in HCA2 cells transduced with *COL6A1* shRNA constructs (sh1 and sh2) or non-targeting control (NTC) constructs, normalised to *GAPDH* and expressed relative to NTC. Western blot analysis of type VI collagen expression in cells transduced with *COL6A1* shRNA constructs (sh1, sh2, and sh3), transduced with NTC constructs, or untreated (UTC). (c) Schematic of procedure for generation of cell-derived matrices (CDMs). (d) Representative immunofluorescence images of

type VI collagen deposition in CDMs generated by NTC and sh1 expressing cells after 10 days in culture. Data represent mean ± S.E.M. (N=6). Scale bars: 25 μm

Figure 3: Type VI collagen mediates ECM assembly. (a) Representative immunofluorescence images of fibronectin (Fn) fibres of CDMs generated by HCA2 cells expressing NTC, sh1, or sh2 shRNA after 10 days in culture. (b) Corresponding histograms of fibre orientation distribution for representative images in panel a. (c) Quantification of total CDM thickness, Gaussian standard deviation of fibre orientation, interfibrillar space and fibre diameter for CDMs produced by NTC, sh1, or sh2 expressing cells. Data represent mean ± S.E.M. (N=3 experiments, 15 images per experiment) *$p<0.05$ and ***$p<0.001$ versus NTC, ###$p<0.001$ versus sh2. (d) Representative images of Fn fibres produced by NTC or sh1 expressing cells at days 3, 5, 7, and 10. (e) SEM images of CDMs at days 5 and 10. Scale bars: 25 μm for a and d and 10 μm for e.

Figure 4: Type VI collagen affects ECM composition. (a) Representative immunofluorescence images of type I collagen, vitronectin, tenascin C, versican, and decorin in CDMs generated by NTC and sh1 expressing cells. (b-e) Quantification of biochemical composition of CDMs, including (b) soluble collagen using the Sircol™ assay, (c) total collagen using the hydroxyproline assay, (d) DNA content using the Hoechst dye assay, and (e) sGAG content using the DMMB assay. Data represent mean ± S.E.M. (N=3 experiments) *$p<0.05$. Scale bars: 25 μm.

Figure 5: Dermal fibroblast morphology is altered on collagen VI depleted CMDs. (a) Fluorescence images of F-actin (green) in primary dermal fibroblasts cultured for 24 hours on CDMs derived from NTC or sh1 expressing cells or on Fn coated coverslips. (b) Quantification of fibroblast number, spread area, and aspect ratio on CDMs or Fn coated surfaces. (c) Immunofluorescence images of α-smooth muscle actin (aSMA, red) and Ki67 (green) expression in primary dermal fibroblasts cultured on CDMs derived from NTC or sh1 expressing cells or Fn coated coverslips for 7 days (no ascorbic acid in medium). (d) Quantification of aSMA and Ki67 positive cells on CDMs and Fn coated coverslips after 7 days. (e) Representive images of primary fibroblasts stained with oil red O and counterstained with haematoxylin following 7 days of culture on CDMs or Fn surfaces. All data represent mean ± S.E.M. (N=3 experiments), *p<0.05, **p<0.01 and ***p<0.001 versus NTC, #p<0.05, ##p<0.01 and ###p<0.001 versus sh1. Scale bars: 25 μm.

Figure 6: Type VI collagen depleted CDMs enhance fibroblast motility. (a) Immunofluorescence images of primary fibroblast morphology (F-actin, green) in relation to Fn fibres (red) of CDMs generated by NTC or sh1 expressing cells. (b) Tracks of individual cells migrating on CDMs or Fn coated tissue culture wells. Cells were imaged using time-lapse phase-contrast microscopy and analysed with ImageJ. (c) Quantification of single cell velocity and persistence (directionality) on CDMs or Fn coated tissue culture wells. Data represent mean ± S.E.M. (N=4 experiments), *p<0.05 and ***p<0.001 versus NTC, ###p<0.001 versus sh1. Scale bars: 25 μm.