

# Collagen matrix wound dressings and the treatment of DFUs

- **Objective:** To obtain clinical evidence on the use of a collagen wound dressing in patients with diabetic foot ulcers (DFUs).
- **Method:** A convenience sample of patients managed in the podiatry outpatient clinic over an 8-month period were included in the evaluation, if their DFUs were not progressing. All participants received traditional adjunctive wound care as part of their treatment. Wound surface-area measurements and imaging of patients was carried out on a regular basis to allow the tracking of wound healing.
- **Results:** Six patients, with seven wounds were included in this study. There was an overall decrease in wound surface area over time. Three patients showed a relatively swift reduction in wound area, while one patient showed a slight increase in wound area. The percentage decrease in wound area levelled out quite quickly in three patients, with a mean wound duration of 14 months compared with 7.3 months in the remaining four wounds. One patient died of an unrelated cause.
- **Conclusion:** This case series evaluation provides a snapshot of experience at one clinical centre and the treatment of DFUs, and suggests that an active biological collagen dressing may support progression to healing by modulating the wound environment.
- **Declaration of interest:** S. Haycocks and P. Chadwick received supplies of ProHeal from MedSkin Solutions Dr Suwelack for use on patients in this evaluation. K.F. Cutting is a consultant to MedSkin Solutions Dr Suwelack and received a honorarium for his participation. MedSkin Solutions Dr Suwelack did not have any editorial control in the production of this manuscript.

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The tissues of the human body may be characterised functionally into two main groups: those that manage bodily processes and undertake maintenance and repair functions, and those that form the major structural component of the body—the extracellular matrix (ECM) tissue. The ECM is primarily fibrous in nature and comprises polysaccharides, such as hyaluronan, and native polymers, such as collagen, elastin, proteoglycans and fibronectin, among others.<sup>1</sup> All of these components are dynamic in nature; they are synthesised, modified and arranged by the cells that they support and sustain. It is important to note that the ECM plays an integral role in daily cellular activity and in tissue regeneration.

Human skin is composed of three distinct layers: the epidermis, which is the outermost layer, the dermis, which lies directly beneath and is separated from the epidermis by the basement membrane, and the subcutaneous layer or hypodermis. Connective tissue within the dermal layer provides skin with its physical strength and function; these attributes of the dermis arise as a result of collagen, which is responsible for up to 80% of the dry weight of the dermis.<sup>2</sup> Dermal collagen is composed primarily of type I (80–85%) and type III (8–11%) collagens.<sup>3</sup>

Within the dermis, the ECM not only provides physical structural support for dermal cells but also

acts as a binding site/reservoir for a host of chemical messengers, growth factors and active molecules that are released following tissue injury to help stimulate cellular migration, recruitment into the wound site and proliferation.<sup>4</sup>

The majority of the ECM is formed of collagen.<sup>2</sup> Collagen is a ubiquitous protein found in connective tissue, blood vessels and the skin. It is synthesised by fibroblasts and has an extensive role to play in the wound healing process, including chemotaxis of fibroblasts and keratinocytes. Collagen is either directly or indirectly involved in each of the four phases of wound healing:<sup>4,5</sup>

- Haemostasis, through platelet activation, is one of collagen's most prominent functions<sup>6</sup>
- During the inflammatory response, collagen influences cellular mitogenesis, differentiation and migration<sup>7</sup>
- A key part of the proliferative phase involves fibroblasts laying down structural proteins of the ECM, including collagen. The cells and the ECM interact closely and continually, stimulating each other during the proliferative phase<sup>4</sup>
- During the final phase of wound healing (maturation/remodelling) fibroblasts continue to lay down collagen.<sup>8</sup> Additional covalent cross-linking of collagen molecules helps to remodel the wound with a final tensile strength of up to 80% of normal, uninjured tissue.<sup>9</sup>

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It is evident that collagen is fundamental to successful wound closure. What is not so clear is the magnitude of collagen's influence within the total repair process.

### Chronic wounds

Wounds that fail to heal in a timely manner and do not effectively respond to orthodox management practices using accepted protocols are classified as 'chronic wounds'.<sup>10</sup> Chronic wounds display a considerably higher level of protease expression than acute wound healing,<sup>11</sup> with elevated levels of proteases,<sup>9</sup> pro-inflammatory cytokines and reactive oxygen species (ROS) found.<sup>12</sup> Conversely, tissue inhibitors of metalloproteinases (TIMPs), which in normal wound healing limit matrix metalloproteinase (MMP) function, tend to be present at lower levels in chronic wounds.<sup>13</sup>

The persisting low level of inflammation, which can typify chronic wounds, results in a reduction in the deposition of the ECM proteins, such as collagen, while any ECM that the fibroblasts do manage to lay down in the wound bed is at a greater than normal risk of being broken down by the unchecked activity of the MMPs.<sup>3</sup> This constant struggle to deposit and maintain a fully-functioning ECM is a problem that needs to be addressed clinically.

Wound treatments with ECM substitutes, such as acellular collagen matrices, look to mimic the structural and functional characteristics of healthy ECM and offer a biological approach to accelerate the healing process.<sup>14</sup> Such matrices can be placed directly onto the wound to help expedite healing by acting as a wound scaffold.

### Mechanism of action

When used in acute situations, such as breast reconstruction or hernia repair, biological implants, such as acellular collagen matrices, are believed to become fully incorporated into the new tissue; however, when used in chronic wounds these matrices never fully integrate into the tissues and are ultimately displaced.<sup>15</sup> It is thought that placement of these acellular biological matrices into the chronic wound bed helps modulate the wound environment by acting as a biological cover, which facilitates wound healing.<sup>16</sup>

While the exact mechanism of action for biological dressings remains unclear, it is thought to be multifaceted. Collagen-based matrices are thought to influence or contribute to wound healing in a variety of ways. These can include the provision of growth factors to the wound bed, maintaining a biochemically-balanced, moist wound environment, and the provision of a physical, structural support to facilitate tissue regeneration.<sup>17</sup> In addition, a number of pre-clinical studies have suggested its benefits in wound healing may also include:

<b>Table 1. Acellular collagen matrices</b>	
<b>Product format</b>	Powder, gel, paste, mesh/sheet, fleece
<b>Collagen source</b>	Human, bovine, equine, porcine, avian, piscine, ovine
<b>Product offering</b>	Collagen only Chemically cross-linked collagen Collagen combined with (silicone membrane, antimicrobial, regenerated cellulose, alginate, synthetic polysiloxane)

- MMP inhibition or deactivation<sup>7</sup>
- Stimulation of angiogenesis<sup>18</sup>
- Act as a chemoattractant for endothelial cells<sup>19</sup> and fibroblasts<sup>20</sup>
- Provide a scaffold, which facilitates fibroblast ingrowth and attachment,<sup>21</sup> and the formulation of granulation tissue<sup>22</sup>
- The protection and/or donation of growth factors to the wound.<sup>18</sup>

Despite the growing knowledge base surrounding collagen and its use in biological dressings, the exact mechanisms of action and the roles played by acellular matrices in chronic-wound healing have yet to be fully understood and defined.

### Collagen in advanced wound care

A considerable variety of collagen-based products currently exist for use in advanced wound care. For simplicity, these products can be broadly categorised as either 'allografts' or 'xenografts'. Allografts are tissue from one animal species that is transplanted into the same animal species; therefore, in this instance, collagen-based matrices derived from human cadaver skin are examples of allografts. Xenografts are tissue from one animal species that is transplanted into a different animal species, such as collagen matrices derived from porcine, bovine or equine sources, which are placed into a human wound.

As part of the tissue engineering process, cells within the harvested tissue are typically removed in order to minimise any potential host immune response, such as immunological rejection. This decellularisation results in an acellular collagen matrix that, as far as possible, retains its original/native structure and function. The greater the compatibility between the acellular collagen matrix and the host ECM, the less risk of an adverse reaction.<sup>23</sup> Currently available acellular collagen matrices can vary widely between manufacturers (Table 1).<sup>24,25</sup>

### Biological wound therapy

It is extremely difficult, if not impossible, to design and manufacture synthetic tissue that can comprehensively replicate the functionality of native tissue. Therefore, it is suggested that collagen-based biological wound therapies consisting of natively-structured collagen are best able to provide the desired functions of promoting cell adhesion, cellular growth and support of cell migration.<sup>26</sup>

ProHeal (MedSkin Solutions Dr Suwelack) is a sterile, biological matrix that comprises of natively-structured bovine collagen fibres, provided as a three-dimensional (3D), open-pore matrix. It is indicated for all wounds healing through secondary intention, where the wound bed is free of devitalised necrotic tissue, including acute traumatic and surgical wounds, and chronic wounds, such as diabetic foot ulcers (DFUs), venous leg ulcers, pressure ulcers and ulcers of mixed aetiology.

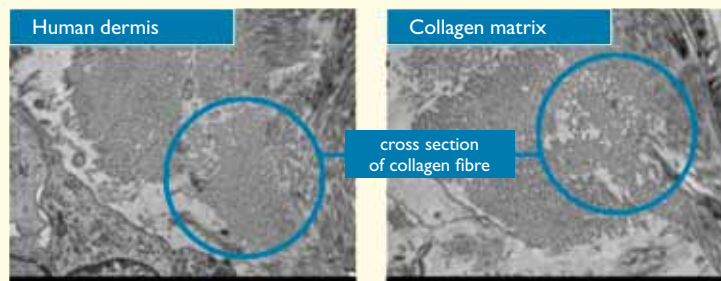
Debridement of the wound bed is recommended prior to application of the collagen matrix. Sharp debridement may result in bleeding of the soft tissue; however, native collagen has demonstrated haemostatic properties,<sup>27</sup> so application to freshly debrided wounds is permitted. Avoidance of chemical cross-linking ensures that the collagen will be resorbed, although residues may remain on the wound bed.<sup>28</sup> The rate of application of the collagen matrix dressing may vary with the volume of exudate produced but on average twice weekly treatment is anticipated.

Interactions between cells and their micro-environment is a feature of the healing process.<sup>29</sup> Responses to such interactions include cellular differentiation, dedifferentiation and proliferation, and lead to the cellular structure and function associated with dermal tissue. Observation of these processes has led to the concept of dynamic reciprocity, encompassing the enduring and bidirectional cellular/ECM interaction.<sup>29</sup> In collagen matrix dressings, native collagen replaces the ECM and also acts as a scaffold for granulation tissue growth and migration.

The close similarity between the native collagen fibrous structure of human dermis and the dressing (Fig 1) lends support to the use of native collagen, where chemotaxis of fibroblasts and keratinocytes will support cellular growth and migration. Wound closure is achieved with complete re-epithelialisation following the migration of keratinocytes over the granulation tissue. The migration of keratinocytes is strongly associated with expression of collagen IV.<sup>30</sup> It is thought that stimulation of collagen IV expression takes place following the diffusion of collagen matrix dressing peptides, potentially accelerating wound closure (personal communication with Dr R. Bauer, Regensburg, Germany).

The aim of this evaluation was to appraise the use of an active biologic wound therapy, a collagen

**Fig 1. Transmission electron microscopy images of structure of human dermis and the collagen matrix dressing**



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wound dressing (ProHeal) on a convenience sample of patients with slow-to-heal DFUs.

### Method

A convenience sample of patients presenting at Salford Royal NHS Foundation Trust, UK and managed in the podiatry outpatient clinic between October 2011 and August 2012 were included in the evaluation, if their DFUs were not considered to be progressing, with the wounds showing little change in appearance in the wound bed and the size remaining static for a period of at least 3 weeks. The aim of the treatment was to 'kick start' healing, where wound healing appeared to have stalled.

### Treatment protocol

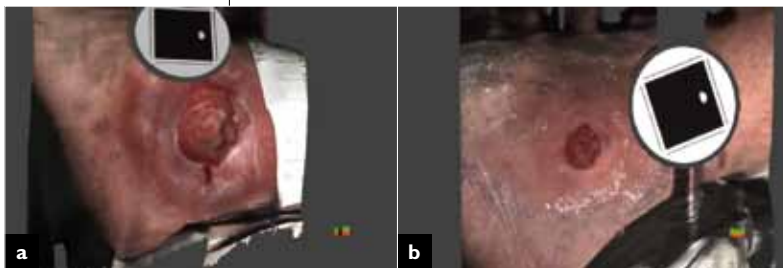
All participants received traditional adjunctive wound care as part of their treatment. Holistic care of the patient focused specifically on pressure relief/offloading of the ulcerated area, appropriate for their foot and wound type. Wound-bed preparation prior to application of the collagen matrix included regular debridement and management of exudate levels. Infection was deemed to be present by recording the following criteria: redness, pain, oedema, heat, swelling, malodour and/or the presence of osteomyelitis.<sup>31</sup> Systemic antibiotics were used concomitantly, if judged necessary by the investigators. Pain was managed through administration of appropriate analgesics.

Dressing changes were undertaken according to local clinical practice (usually three times per week). Patients were seen once a week by podiatry services, with other dressing changes carried out by district nurses or the patient themselves.

The collagen matrix dressing was applied directly over the entire wound bed. In wounds with little or no exudate, the dressing was moistened with normal saline. Secondary dressings used were Melolin (Smith & Nephew), Allevyn adhesive (Smith & Nephew), Biatain (Coloplast) and sorbion sachet S (sorbion), to maintain a moist wound environment.



**Fig 2.** Patient 5, showing the DFU of 2 months' duration at study start (a) and after 3 weeks' treatment with the collagen matrix wound dressing (b)



**Fig 3.** Patient 6 presented with a wound containing about 30% slough at the start of treatment (a); this devitalised tissue may account for the modest decrease in wound area (b)

At dressing change, any residual collagen matrix was left in the wound. Patients received the collagen matrix dressing, until application was stopped at the discretion of the lead clinician.

The Eykona wound measurement system was used to provide accurate and repeatable measurement of wounds throughout the evaluation. This system has been evaluated in clinical studies of diabetic ulcers and shown to have very low intra- and inter-assessor variability.<sup>32,33</sup> In this evaluation it was used to provide wound surface-area measurements. Imaging of patients was carried out on a regular basis to allow the tracking of wound healing. Once acquired, image data were assessed locally. Data can also be assessed remotely, immediately or retrospectively to allow for additional measurement information to be acquired.

### Results

Six male patients, all with type II diabetes mellitus and peripheral neuropathy were included in the study. Three patients were diagnosed with peripheral arterial disease. The median age was 59 years (range 54–82 years). One patient had a foot ulcer on both his left and right feet. The median wound duration was 12 months (range 2–18 months). Exudate levels were low in two patients and moderate in four patients (five wounds).

Patients received a variety of dressings prior to treatment with the collagen matrix dressing. These included various polyurethane foams, Hydrofiber, Mextra (Mölnlycke), sorbion sachet s (sorbion),

Biatain (Coloplast), Kerramax (Crawford Healthcare), Granugel (ConvaTec), Flaminal (Crawford Healthcare), Actisorb Silver 220 (Systagenix), Inadine (Systagenix), Aquacel (ConvaTec), Acticoat Absorbent (Smith & Nephew), negative pressure wound therapy (NPWT) and larvae debridement therapy (LDT).

- **Patient 1** an 82-year-old male presented with a DFU of 12 months' duration on his left foot, with moderate wound exudate. Treatment prior to collagen matrix included NPWT, foam dressings, antimicrobial dressings, and intravenous (IV) antibiotics vancomycin and piperacillin/tazobactam for osteomyelitis. Adjunctive therapies included Darco's orthopaedic shoe with a total contact insole and weekly debridement.

- **Patient 2** a 58-year-old male with a left foot DFU of 12 months' duration, with moderate wound exudate. Prior treatment included NPWT, superabsorbent dressings, and IV antibiotics meropenem and daptomycin for extensive osteomyelitis in the calcaneus. Adjunctive therapies included ROM Walker and weekly debridement.

- **Patient 3** a 59-year-old male with DFUs on both his left and right feet, of 12 months' duration. Both ulcers had a moderate level of exudate. Prior treatment included antimicrobial dressings, foam dressings and a superabsorbent dressing. Adjunctive therapies included bilateral slipper casts and Derby sandals.

- **Patient 4** a 55-year-old male with a DFU on his right foot of 18 months' duration, with moderate level of wound exudate. Previous treatment included superabsorbent and antimicrobial dressings. Adjunctive therapies included ROM Walker and weekly debridement.

- **Patient 5** a 54-year-old male with a DFU on his right foot of 2 months' duration (Fig 2a), and a low level of wound exudate. Previous treatment included a wound contact layer and antimicrobial dressing. Adjunctive therapies included slipper cast and Derby sandals.

- **Patient 6** a 74-year-old male with a DFU on his left foot of 3 months' duration (Fig 3a), with low wound exudate. Prior treatment included a hydrocolloid-based gel, wound contact layer, antimicrobial dressing, LDT, and oral antibiotics clindamycin and ciprofloxacin for osteomyelitis. Adjunctive therapies included Derby sandals with total contact insole.

### Outcomes

An overall decrease in wound surface area over time was observed following introduction of the collagen matrix dressing, with three patients (3, 5 and 6) showing a relatively swift reduction in wound area (Fig 2b; Fig 4). Wound volume was not formally measured in this evaluation. Although patient 4 showed a slight increase in wound area, it was esti-



mated that wound volume decreased as the wound was judged to be shallower at day 65 compared with day 1 of the evaluation process. The slight increase in wound area is most likely a consequence of the patient's early return to work and non-concordance with the ROM walker.

The collagen matrix dressing is intended for use on wounds where the wound bed has been adequately prepared and is devoid of any devitalised tissue. One patient (patient 6) wound contained 30% slough at the start of treatment. This devitalised tissue may have been responsible for the relatively slow progress made with this wound and account for the modest decrease (20%) in wound area (Fig 3b).

All other wounds appeared to have a healthy wound bed with granulation tissue of 100%. Sadly, patient 6 died of an unrelated cause at day 61 of the evaluation.

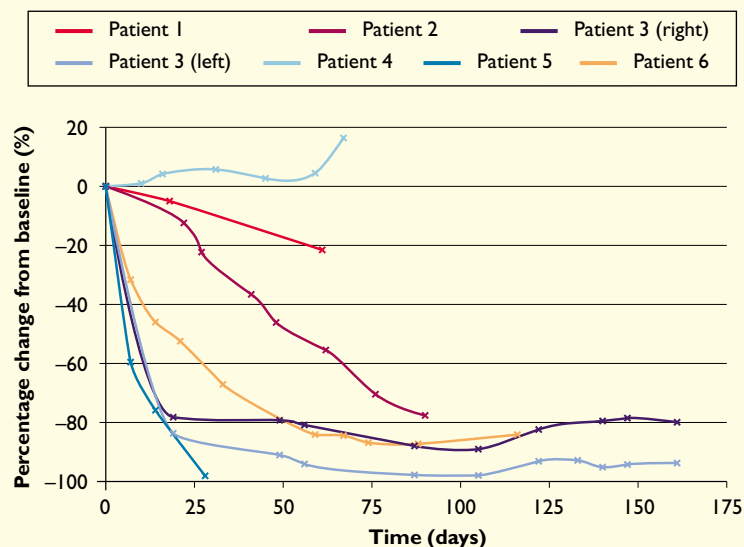
When examining the percentage change in wound area over time, the cohort of patients appear to divide into two distinct groups (Fig 4). The percentage change in wound area in patients 1, 2 and 4 levels out quite quickly. These three patients' wounds had an average duration of 14 months compared with the other four wounds, whose average duration was 7.25 months. This supports the observation that longer standing wounds are slower to progress. Patients 3 (left and right), 5 and 6, whose wounds showed a higher percentage change in area, had all received a topical antimicrobial dressing prior to commencing treatment with the collagen matrix. Patient 5 healed after 7 weeks' treatment.

## Discussion

Management of stalled wounds is a challenge faced by clinicians on a regular basis. It is estimated that 15% of diabetic patients develop a neuropathic foot ulcer and 15% of these patients will eventually undergo an amputation.<sup>34</sup> Recurrence of foot ulcers in patients with diabetes is high,<sup>35</sup> and mortality rates in diabetics with a history of foot ulceration can be up to 49%.<sup>36</sup> The challenge is to find an effective treatment that will close the ulcer in a timely fashion, to avoid the risk of infection and associated morbidity. The patients recruited to this evaluation were considered to be representative of DFU patients encountered in a hospital outpatient clinic. The results of this small-scale evaluation suggest that the collagen matrix dressing is suitable for application in DFUs when the wound bed has been adequately prepared.

In a clinical investigation of 40 patients comparing ProHeal with standard therapy in patients with venous leg ulcers, Romanelli and colleagues<sup>37</sup> found that 85% of patients treated with the collagen matrix dressing showed a greater than 50% increase in granulation tissue formation at 4 weeks and con-

**Fig 4. Percentage change in wound area over time**



cluded that ProHeal was safe and efficacious in hard-to-heal venous leg ulcers when compared with the control group.

## Limitations

The evaluation comprised of a series of six patients with seven DFUs recruited as a convenience sample, with no inclusion or exclusion criteria applied. Data collection ceased when application of the collagen matrix dressing was stopped, at the discretion of the lead clinician. Therefore, the longer-term implications of wound resolution following application of the dressing cannot be assessed. Nonetheless, the data collection does capture real-world experience of a collagen matrix and avoids the artificiality that is imposed by a randomised controlled trial.

## Conclusion

Although our understanding of the role of collagen in the skin and in the repair process has been described, comprehensive insight has yet to be acquired. Collagen matrices have a number of potential roles to play in the repair process including; act as a scaffold to support tissue formation, chemotaxis of endothelial cells, stimulation of angiogenesis, promote fibroblast attachment, provide and protect growth factors and to promote the normal wound healing process by modulating the wound environment. This case series evaluation provides a snapshot of experience at one clinical centre. It is recommended that a more robust case cohort investigation into the impact of the collagen matrix dressing in the management of DFUs is undertaken. ■

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