

# The use of amelogenin protein in the treatment of hard-to-heal wounds

Paul Chadwick, Claire Acton

## Abstract

The management of hard-to-heal or chronic wounds places a high economic burden on healthcare services. This problem is exacerbated by the increasing age of the general population, an increasing diabetes population and a high prevalence of such wounds in the elderly, patients with diabetes and those with venous insufficiency. Standard treatments for such wounds, such as compression therapy in venous leg ulcers, debridement and wound care for diabetic foot ulcers, can still leave a significant population with non-healing wounds, resulting in extended hospital stays and reduced quality of life. The use of amelogenin (Xelma®, Mölnlycke Health Care) for the treatment of a variety of chronic wounds has been assessed in both case studies and larger clinical trials with encouraging findings. This article examines the findings of studies relating to amelogenin in the treatment of hard-to-heal wounds.

**Key words:** Amelogenin ■ Extracellular matrix ■ Hard-to-heal wounds

By definition, chronic or hard-to-heal wounds are those that are slow to heal. Typically, they have a duration of more than four weeks (Clarke-Moloney et al, 2005) and are characterized by the failure to progress through the normal stages of wound healing seen in acute wounds, which are defined as lesions of short duration that heal according to a defined process (Menke et al, 2007) (Figure 1). A holistic approach to the management of a patient presenting with a difficult wound should include ensuring glucose control (in the case of patients with diabetes), determining that the blood supply is adequate, ensuring infection is absent and that the wound has been properly debrided, and ensuring that the use of appropriate off-loading is in place. If the wound still fails to heal, then the possible reasons for delayed healing (outlined in Table 1) should be considered.

It is estimated that approximately 1–2% of the population will suffer from a chronic wound, such as a leg ulcer, during their lifetime (Anderson, 2006), and Nelson et al (2006) report the prevalence of such ulcers to be increasing with age

Paul Chadwick is Principal Podiatrist, Salford PCT, Podiatry and Foot Health, Hope Hospital, Salford, UK and Claire Acton is Tissue Viability and Vascular Nurse Specialist, Surgical Directorate, Queen Elizabeth Hospital NHS Trust, London, UK

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to approximately 20 per 1,000 of the population at the age of 80 years. Examples of typical chronic wounds are venous leg ulcers, diabetic foot ulcers, pressure ulcers and burns.

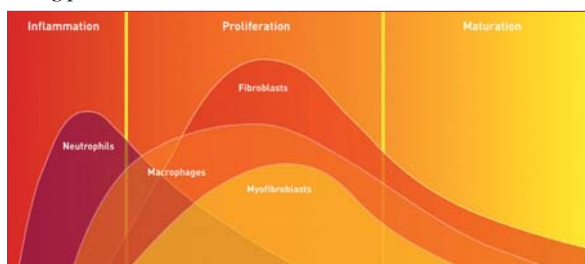
Chronic ulceration of the lower leg is common. Approximately 3–5% of the population over 65 years of age will suffer from a leg ulcer during their lifetime (Mekkes et al, 2003). The majority of leg ulcers result from venous insufficiency (45–60%), arterial insufficiency (10–20%), diabetes (15–25%), or combinations of these aetiological factors (Mekkes et al, 2003). Hard-to-heal pressure ulcers and burns also contribute to the numbers of chronic wounds. In the case of venous leg ulcers, the European Wound Management Association (EWMA) defines them as those ulcers greater than 10cm<sup>2</sup> in size and/or older than six months (EWMA, 2003).

In patients diagnosed as having diabetes mellitus, the prevalence of foot ulcers is 4–10%, while the annual population-based incidence is between 1.0 and 4.1% – the lifetime incidence may be as high as 25% (Singh et al, 2005). These data indicate that a high percentage of foot ulcers are chronic. Amputation is associated with a significant rise in mortality at follow-up, ranging from 13–40% at one year to 39–80% at five years (Singh et al, 2005).

The economic impact of diabetic foot ulcers is extremely high, being reported by Lithner (1992) to be responsible for 47% of all diabetes-related hospital admissions. An estimated 7–20% of total expenditure on diabetes is thought to be attributable to diabetic foot disease (Boulton et al, 2005).

The economic impact of chronic wounds on the health-care sector is huge, with the ageing population set to increase dramatically. In the US it is estimated that over \$3bn per year is spent on the treatment of these lesions. In general, chronic wounds are very painful and have a detrimental impact on patients' quality of life (Price et al, 2007). Additionally, stress caused by wound-related pain is thought to further delay the wound-healing process (Soon and Acton, 2006).

Figure 1. The stages of wound healing and temporal changes of major cell types (neutrophils, macrophages, fibroblasts, myofibroblasts) involved in the healing process.



**Table 1. Reasons for delayed healing**

- Growth factor imbalance
- Extracellular matrix deficiency compromising tissue remodelling and re-epithelialization
- Excessive or uncontrolled proteinase activity
- Senescent cells

Reported healing rates for these types of wounds vary across studies. In one diabetic foot ulcer study in which patients were treated with standard care, a 79% healing rate was attained at 25 weeks (Piaggese et al, 1998). In contrast, Margolis et al (2005) have recorded healing rates in diabetic foot ulcers to be as low as 24% at 12 weeks and 31% at 20 weeks. About 20–30% of venous leg ulcers are unresponsive to standard treatment with compression therapy (Barwell et al, 2004; Margolis et al, 2005), and in some cases more than 20% of venous leg ulcers have been reported as failing to heal after 70 weeks, despite the use of compression therapy (Rippon et al, 2007). As a result, these so-called recalcitrant lesions may require the use of advanced therapies to stimulate tissue repair (Silhi, 1998; Rippon et al, 2007), including:

- Growth factors applied as a gel formulation to the wound bed
- Protease scavenger devices that remove proteases from the wound bed while returning undamaged growth factors (Promogran®, Systagenix)
- Negative pressure therapy (e.g. VAC®, KCI; Vista®, Smith & Nephew) which increases blood flow at the wound bed, reduces bacterial burden and stimulates granulation
- Artificial skin grafts
- Extra-cellular matrix substitutes such as amelogenin (Xelma®, Mölnlycke Health Care).

Xelma is an advanced therapy, recommended for the treatment of hard-to-heal wounds and containing the protein amelogenin – this is an extra-cellular matrix biocompatible protein that, when applied to the wound bed, provides a surrogate extra-cellular matrix or scaffold upon which cells involved in the healing process, such as dermal fibroblasts, can adhere, migrate, proliferate, and synthesize chemical signalling messengers and new-matrix components, thus promoting the wound healing process.

*Figure 2. Cell adhesion to amelogenin matrix protein. Scanning electron micrograph of a dermal fibroblast bound to two protein aggregates (courtesy of Professor Bengt R Johansson).*



In physiological conditions, amelogenin self-assembles into globular aggregates of up to micron-size that effectively form the temporary apparatus required to promote wound healing while restoration of the normal balance of growth factors and proteases is achieved (Figure 2). This enables advancement to the proliferative phase of the healing process.

### Clinical studies

Outside its use in the treatment of periodontal wounds, the greatest clinical experience with amelogenin to date has been in the treatment of venous leg ulcers. However, only two case series have considered its use in the treatment of diabetic foot ulcers, and another has looked at its use in the treatment of pyoderma gangrenosum. In the case of venous leg ulcers, amelogenin has been successfully used in the treatment of ulcers that have failed to respond to standard therapy with compression. This article looks at the findings made in the clinical and smaller case studies conducted with a variety of chronic wound types.

### Diabetic foot ulcers

Meuleneire et al (2007) evaluated amelogenin in a study consisting of a mixed population of patients with diabetic foot ulcers (n=5) and venous leg ulcers (n=5). Patients were treated with weekly applications of amelogenin for 12 weeks. Overall, 80% of ulcers that had previously failed to heal changed from a static to a healing state, signifying that amelogenin had a beneficial effect in the management of these wounds (Meuleneire, 2007).

### Venous leg ulcers

Romanelli et al (2006) undertook a study to determine the optimum number of applications of amelogenin required to produce a healing response in patients with hard-to-heal venous leg ulcers of more than six months duration. Patients were treated with amelogenin once a week in conjunction with high compression therapy. Patients receiving 12 weeks of treatment had a larger reduction in ulcer size (-72%) than those receiving treatment for three weeks (-22%) or six weeks (-48%), respectively (Romanelli et al, 2006).

In a single-blind, multicentre study designed to assess the effect of amelogenin on hard-to-heal venous leg ulcers, 123 patients were randomized to receive either amelogenin in combination with high compression therapy (n=62) or a control treatment consisting of a placebo gel (amelogenin carrier vehicle) plus high compression bandaging (n=61). Although the percentage wound size reduction was greater in the treatment group, no statistically significant differences were detected between groups. However, analysis of those patients with ulcers greater than 10cm<sup>2</sup> at baseline and of duration greater than 12 months showed a difference in percentage wound reduction in the active treatment group (33.8% versus 25.6%). Because of the small sample numbers used in this sub-group analysis, the differences between treatment arms did not reach significance.

Vowden et al (2007a) conducted a trial that compared amelogenin plus compression bandaging, with compression therapy alone in the treatment of non-infected venous leg ulcers of more than six months duration and with a surface

area of 10–30cm<sup>2</sup>. The study concluded that amelogenin used as an adjunct to compression therapy results in:

- A significant reduction in ulcer size
- Reduced pain between visits and at dressing changes
- A larger proportion of ulcers with no or low levels of exudate.

In this open, randomized, comparative, parallel group, multicentre investigation, 83 patients were allocated to receive either active treatment (n=42) or compression therapy alone (n=41). The study was conducted over 24 weeks, which included 12 weeks follow-up after the final treatment at 12 weeks (Romanelli et al, 2008; Vowden et al, 2007a). Compared with the control group, the amelogenin group had the greatest percentage reduction in ulcer size from baseline to the last assessment visit at 12 weeks (-33.1% versus -11.07%) (Figure 3) (Vowden et al, 2007a; Romanelli et al, 2008). The number of improved ulcers was significantly greater in amelogenin-treated patients than controls.

A larger number of wounds with reductions in size of greater than 50% were noted in the amelogenin group – in

the control group more wounds showed an increase in ulcer size. The overall healing rate was found to be greater in the amelogenin-treated group. At the last assessment visit the percentage of improved ulcers was statistically significantly higher in the amelogenin group (47.5% versus 19.5%; P=0.01) (Figure 4).

Statistically significant differences in favour of amelogenin-treated patients were also found for:

- Reduction in ulcer-related pain (P=0.01)
- Reduction in pain at dressing changes (P=0.02)
- Proportion of patients with none or only low levels of exudate (P=0.01) (Vowden et al, 2007a; Romanelli et al, 2008) (Figures 5–7).

A series of photographs of a venous leg ulcers patient treated with amelogenin is shown in Figure 8. The patient was female, Caucasian, 83 years of age, and presented with a venous leg ulcer that was eight months old on her left leg (Vowden et al, 2007a).

Romanelli et al (2008) comment on the fact that the patient population evaluated in this study represents the ‘worst case scenario’, with some of the patients in the amelogenin group having ulcers which were 10 or more years old. This emphasizes the potential advantages of using advanced therapies, such as amelogenin, for the treatment of ulcers within this patient population. The results of Vowden et al’s (2007a) study also indicate the favourable safety profile of amelogenin, which was associated with few adverse effects and no significant differences between the control and treatment groups in this respect (Romanelli et al, 2008).

The findings of Vowden et al’s (2007a) study are in agreement with other clinical studies (Vowden et al, 2006; Huldt-Nystrom et al, 2007; Huldt-Nystrom et al, 2008), which have demonstrated that the addition of amelogenin to high compression bandaging is statistically and clinically beneficial to the healing of hard-to-heal venous leg ulcers in regard to reducing ulcer size, reducing pain between visits and at dressing changes, and achieving a higher proportion of ulcers with none or low levels of exudate.

Huldt-Nystrom et al (2007) used case studies to assess the effects of amelogenin on healing parameters in hard-to-heal venous leg ulcers classified as those of a duration greater than six months and having a surface area exceeding 10cm<sup>2</sup>. In one of these case series, the findings from 19 patients with a total of 25 non-healing venous leg ulcers were assessed. Amelogenin was applied to a clean wound bed every week for a maximum of 12 weeks and patients also received compression bandaging. In order to ensure that ulcers would not have responded to compression therapy alone, most patients had been treated with compression bandaging for four weeks before inclusion in the study. Overall, 76% of the wounds either healed or reduced in size after treatment. Nine ulcers (36%) healed, while 10 (40%) showed a significant improvement. Approximately one-quarter of the wounds failed to improve and either remained unchanged (n=3; 12%) or deteriorated (n=3; 12%) during the treatment period. In wounds with high levels of exudate, skin maceration developed along with additional ulceration. These observations support the prescribing information for Xelma, which indicates that amelogenin

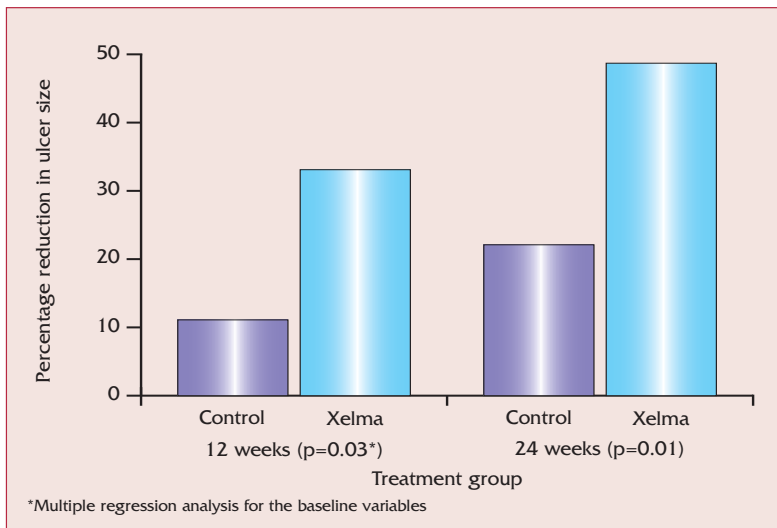


Figure 3. Percentage reduction in ulcer size at 12 and 24 weeks (Vowden et al, 2007a).

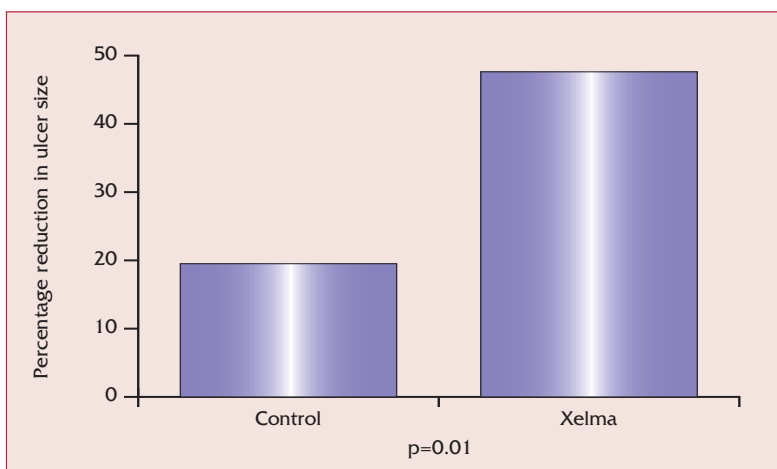


Figure 4. Percentage of patients with 50% or greater reduction in ulcer size at 12 weeks (Vowden et al, 2007a).

is contraindicated in highly exuding or infected wounds (Huldt-Nystrom et al, 2008). This is because high levels of wound exudate wash the amelogenin out of the wound bed – it is estimated that amelogenin requires a minimum of 15 minutes exposure to the wound bed for it to be effective (Huldt-Nyström et al, 2007). Therefore, treatment of infection, inflammation and heavy wound exudate

before application of amelogenin is vitally important for the achievement of an optimal effect.

In this case series there were also occasions when patients reported alleviation of pain during treatment, which supports, along with the reductions in wound size, the findings of Vowden (2006; 2007a).

Another study has demonstrated a healing response to amelogenin in patients with hard-to-heal venous leg ulcers, and although healing was incomplete at the end of the treatment period, the wounds were healthier than before implementation of the treatment. Additionally, quality of life was reported to have improved in these patients (Acton, 2007).

In another case study series, eight patients with a total of 10 venous leg ulcers (mean duration of 9.3 years), each received an application of amelogenin. The duration of treatment was 12 weeks and participants were followed up for a further 24 weeks. A 50% overall healing rate was noted and the remaining wounds were in a healing state at 24 weeks. Compared with baseline values there was a mean reduction in size of 60%. Exudate levels and wound odour were also reduced over the treatment period (Hampton et al, 2007).

### Pyoderma gangrenosum

An inflammatory ulcerative condition known as pyoderma gangrenosum, which causes deep ulcers to occur on the legs, has been shown to respond to treatment with amelogenin. In one case series, two female patients with recalcitrant pyoderma gangrenosum of a mean 11 months duration were treated weekly with amelogenin under an occlusive dressing for a maximum of eight weeks (Dini et al, 2007). The patients were also receiving systemic immunosuppressive therapy both before and during treatment with amelogenin. Improvement in the ulcers was noted, as demonstrated by the increased granulation tissue formation and wound size reduction. As in other studies of venous leg and diabetic foot ulcers, reductions in wound pain were also reported (Dini et al, 2007).

### Other chronic wounds

Vowden et al (2007b) undertook a study involving 17 patients designed to determine the efficacy of amelogenin in the management of a variety of complex hard-to-heal chronic wounds. These included rheumatoid ulcers (n=2), wounds complicated by rheumatoid arthritis (n=3), neuropathic foot ulcers (n=4), venous ulcers (n=4), and a single ulcer of mixed aetiology. As in other studies both wound pain and exudate levels were reduced. In total, six wounds healed after a mean of eight applications of amelogenin (range 3–16 applications). Additionally, a further six patients continuing with amelogenin demonstrated an improvement of a greater than 50% reduction in ulcer size and two patients discontinued treatment due to infection and wound deterioration (Vowden et al, 2007b).

### Conclusion

In a large trial with venous leg ulcers, amelogenin, an extracellular matrix substitute, has been demonstrated to cause significant reductions in ulcer size, reduce wound-associated pain and reduce levels of exudate. These findings are supported by smaller studies on venous leg ulcers, diabetic

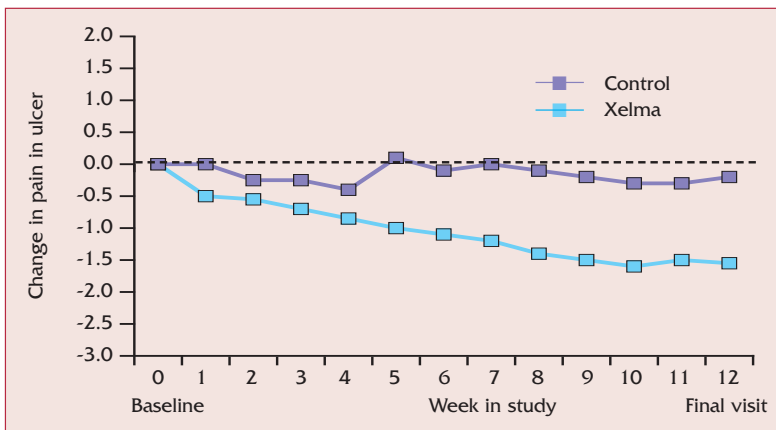


Figure 5. Mean change in pain in ulcer per treatment and week (Vowden et al, 2007a).

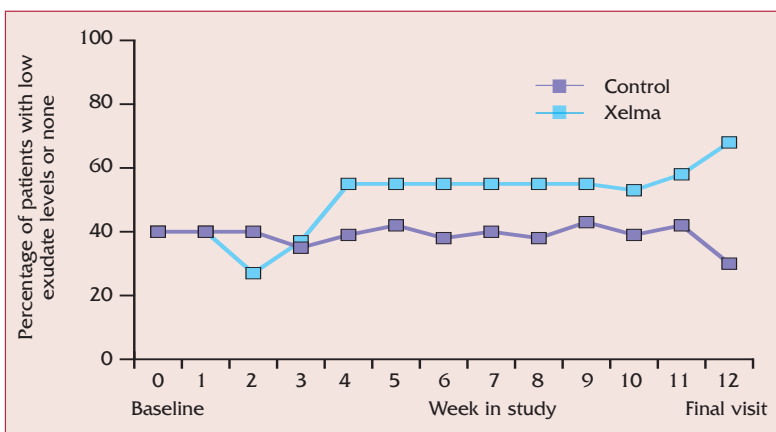


Figure 6. Percentage of patients with low levels of exudate or no exudate in the amelogenin and control groups per week ( $p=0.01$  at week 12) (Vowden et al 2007a).

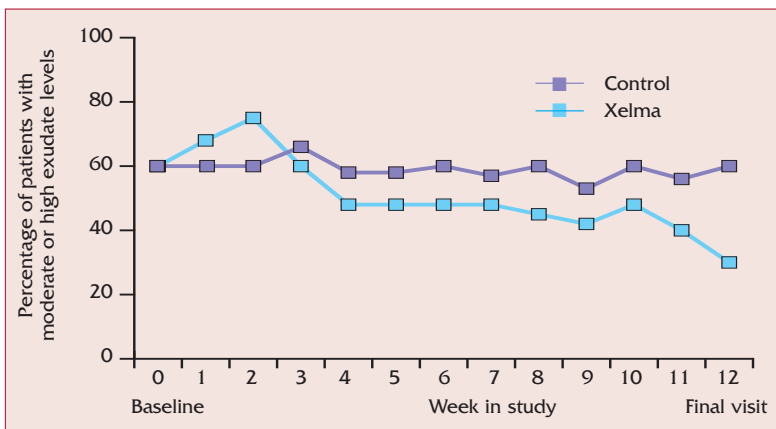


Figure 7. Percentage of patients with moderate or high exudate levels in the amelogenin and the control groups per week (Vowden et al 2007a)



Figure 8. The healing progress of a patient treated with amelogenin over a 12-week period (Vowden et al, 2007a).

foot ulcers, and a variety of other hard-to-heal wounds. It is notable that wounds of a duration greater than six months and of an area exceeding 10cm<sup>2</sup> show the best response rates to treatment with amelogenin.

An independent, global, email survey on hard-to-heal wounds recently conducted at the University of Worcester revealed that 79% of respondents, the majority of which were nurses (83%), declared that they treat hard-to-heal venous leg ulcers or diabetic foot ulcers, indicating that these lesions represent a significant proportion of their caseload (White, 2008). As more advanced treatment options become available to nurses, the implications for improving outcomes in patients with hard-to-heal wounds and the positive impact that advanced treatments, such as amelogenin, can have on the costs of treating these patients needs to be taken into account. An awareness of the advanced treatments now available and the evidence supporting their efficacy should be maintained by nurses involved in wound care. **BJN**

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**KEY POINTS**

- Hard-to-heal wounds are characterized by high levels of proteases, low levels of growth factors, and a compromised extracellular matrix.
- Amelogenin protein is an advanced treatment applied to the wound bed that behaves as a temporary or surrogate extracellular matrix.
- Amelogenin in combination with pressure therapy has been shown to significantly reduce the size of venous leg ulcers, levels of wound exudate, and wound-associated pain.