

Amelogenin: extracellular matrix protein for the treatment of hard-to-heal wounds

Amelogenin is an extracellular matrix protein with biological activity in the regeneration of the periodontium and repair of the skin. Recent published studies have highlighted the use of Xelma® (Mölnlycke Health Care AB, Göteborg, Sweden) in the treatment of hard-to-heal wounds, such as venous leg ulcers. This article reviews the literature relating to the cellular interactions of amelogenin and collates the clinical and health economic data relating to clinical and health economic studies involving patients with chronic wounds. It also discusses a hypothetical mechanism of action of amelogenin in the healing of such wounds.

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KEY WORDS

Amelogenin protein
Chronic wounds
Phases of wound healing
Pain
Exudate

This review explains the essential features of chronic wound formation and goes on to look at the clinical findings made in patients treated with amelogenin protein (Xelma®, Mölnlycke Health Care), a novel advanced treatment that is applied to the wound bed to expedite healing. After considering key clinical studies in which venous leg ulcers (VLUs) have clearly benefited from management with amelogenin therapy (Romanelli et al, 2008a; Chadwick and Acton, 2009; Guest et al, 2009), the author places special emphasis on the findings of recent case studies in which patients with a long history of chronic wounds that remain recalcitrant with conventional therapy have shown marked improvement following treatment with amelogenin, as characterised by increased granulation tissue formation, reduced levels of exudate and abolishment of, or improved management of wound-associated pain.

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How chronic wounds differ from acute wounds

Chronic wounds differ from acute wounds in that they suffer from an imbalance at the level of the wound bed of the normal enzymes and growth factors that are involved in the orderly series of events leading to wound healing. Enzymes that break down proteins are called proteases and these are important in the first phase of the wound-healing process, the inflammatory phase of healing. In the inflammatory phase, these enzymes are effectively debriding the wound of dead (necrotic) tissue, so making the lesion ready for the next phase in wound healing, the proliferation phase. The proliferation phase is followed by the remodelling phase of healing. Essential to the proliferation and remodelling stages is the presence of an intact and functional scaffolding or support structure, on to which cells important to the healing process can attach. This support structure is called the extracellular matrix (ECM).

In wounds that are hard-to-heal because of suspension in the inflammatory phase of the wound-healing process, high levels of proteases in the wound bed result in degradation of natural growth factors (called endogenous growth factors), required for formation of granulation tissue and degradation of the ECM.

Figure 1 summarises the essential phases of wound healing and how these are disrupted leading to the formation of chronic wounds.

Amelogenin proteins

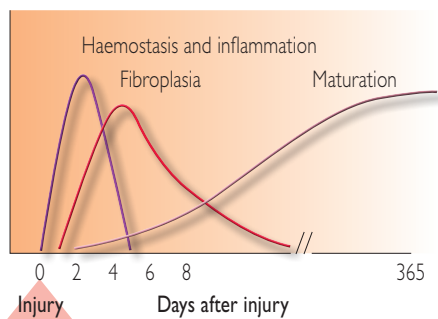
A new approach to rectify the detrimental effects of ECM destruction by excess proteases in the wound bed has been the development of amelogenin proteins (Xelma®, Mölnlycke Health Care). Xelma serves as a temporary or surrogate ECM which acts as a support structure on to which cells, such as those that make collagen (dermal fibroblasts), can attach during the regenerative process of wound healing. This support system enables chronic wounds to be 'kick-started' out of the inflammatory stage of healing into the proliferative and remodelling stages.

Clinical evidence

Xelma has been assessed in both case studies and larger clinical investigations with encouraging findings. To date, the greatest experience with amelogenin has been in the treatment of VLUs (Vowden et al, 2007a; Romanelli et al, 2008; Vowden and D'Arcy, 2008). In one well controlled clinical investigation (Romanelli et al, 2008b), amelogenin has been demonstrated to:

- ▶▶ Increase healing rates
- ▶▶ Reduce ulcer-associated pain
- ▶▶ Reduce levels of wound exudate.

The encouraging findings from this investigation are mirrored in less scientifically robust case study reports ('low-level evidence'), which support the efficacy of amelogenin protein in the treatment of patients with diabetic foot ulcers (DFUs) (McCardle et al, 2009),



The three phases of wound healing in acute wounds:

1. Haemostasis and inflammation
2. Proliferation (fibroplasia)
3. Remodelling (maturation)

Chronic wounds are effectively suspended in the inflammatory stage of the healing process due to imbalances between:

- ▶▶ proteases (essential in the inflammatory phase)
- ▶▶ growth factors (essential for the proliferative and remodelling phases).

Proteases degrade growth factors and the ECM essential for the wound-healing process to progress beyond the inflammatory phase.

Figure 1. The essential phases of wound healing.

and a condition known as *pyoderma gangrenosum* (Berone et al, 2007). In addition, amelogenin therapy has been successfully used in the treatment of a variety of wound types of diverse aetiology, the main findings of which are summarised in this review (Vowden et al, 2007b).

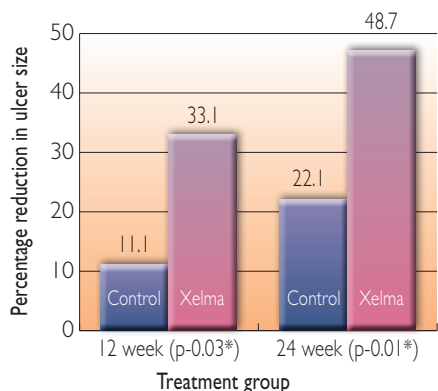


Figure 2. Percentage reduction in ulcer size at 12 and 24 weeks (Vowden et al, 2007a).

Summary of higher level studies

From a clinical investigation (Vowden et al, 2007) involving 83 patients comparing treatment with Xelma plus compression bandaging (n=42), with compression therapy alone (n=41) over a 12-week period in the treatment of VLUs of more than six months' duration, with no clinical signs of infection and with a surface area at inclusion to the trial of 10–30cm², it was demonstrated that when used as an adjunct to compression therapy, Xelma results in:

- ▶▶ A significant reduction in ulcer size (p=0.03) (Figure 2)
- ▶▶ Improvement in the state of ulcers (p=0.01).

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

- ▶▶ Reduction in ulcer-related pain (p=0.01) (Figure 3)
- ▶▶ Reduction in pain at dressing changes (p=0.02)
- ▶▶ Proportion of patients with no or only low levels of exudate (p=0.01) (Figure 4) (Romanelli et al, 2008; Vowden and D'Arcy, 2008).

Figure 5 shows the progress of healing of a VLU treated with Xelma and compression bandaging. The patient was female, Caucasian, 83 years of age, and presented with a VLU of eight months duration on her left leg (Vowden et al, 2007a).

Notable case studies illustrating beneficial effects of amelogenin protein in the management of a variety of wound types

Bond et al (2009) have recently published the findings of three case studies centred on patients with wounds of long-standing duration, which have been characterised by multiple problems and a consequent detrimental effect on patient quality of life. The duration of the wounds and the lack of success in treating them with more conventional means has resulted in excessive cost to the healthcare system when compared with the notably improved results following treatment with amelogenin protein. These case studies are considered briefly here, but a common feature with each study is the improvements noted with respect to granulation tissue formation, exudate

control, and improved pain management when undergoing treatment with amelogenin protein (Bond et al, 2009).

In the first study, a 49-year-old female patient diagnosed with basal cell carcinoma (BCC) underwent an operation in which the carcinoma was removed, resulting in a large area of tissue at the base of the skull becoming exposed. The patient was referred to plastic surgeons for a rotation flap to cover the wound area. However, the distal flap became ischaemic and failed, leaving a large deficit. Management of the failed flap with sharp debridement and the application of Mesitran® (Unomedical) ointment to remove slough was continued over the course of time, with the application of a variety of different wound dressings, including Betadine® ointment with Mepitel® (Mölnlycke Health Care) and furicine Aquacel® Ag (ConvaTec) to treat the exposed wound area. However, there were no signs of granular tissue formation using this treatment protocol. In an attempt to encourage granular tissue formation using Promogran Prisma® (Systagenix Wound Management) an initial response was noted, but the patient found the product painful when applied, and the wound later became infected with exudate levels becoming problematic.

In a further attempt to manage the now infected wound Mepilex Ag was used with initial good response; however, no real foundation of granular tissue was visible. Negative pressure therapy (NPT) could not be used as the affected area was too painful to touch and there would also be difficulties in managing the seal in this area.

The patient then developed a spontaneous haematoma and had two wound infections after this event which were treated with a topical antimicrobial agent plus antibiotics (flucloxacillin). The patient was reluctant to undergo any further grafting because of the pain and poor take of previous attempts.

Treatment with Xelma resulted in initial improvement in the early stages and, although improvement of the wound slowed, it still continued to progress. The tissue and wound bed area were considered by the treating physician to be more robust and healthier than

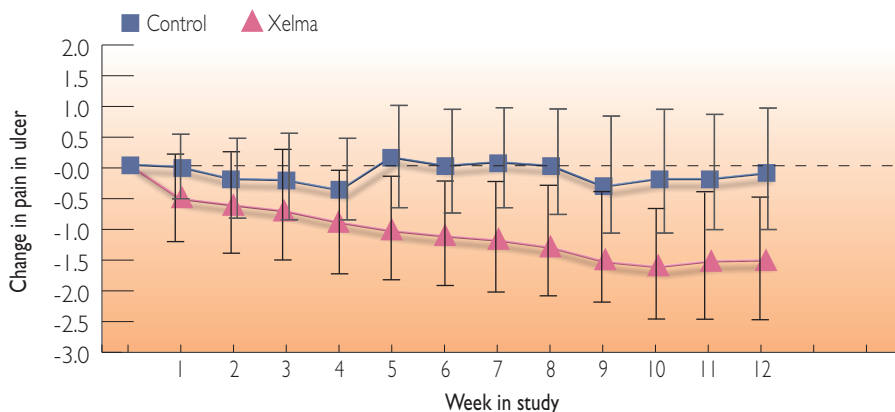


Figure 3. Mean change in pain in ulcer per treatment and week ($p=0.01$) (Vowden et al, 2007a).

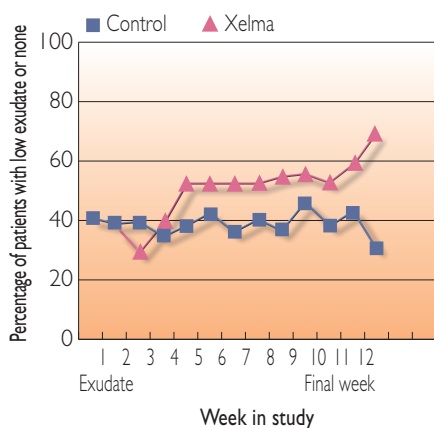


Figure 4. The percentage of patients with low levels of exudate or no exudate in the amelogenin and control groups per week ($p=0.01$ at week 12) (Vowden et al, 2007a).

before treatment with Xelma. A further complication arose when a haematoma developed following the patient banging her head on a car door. The wound became infected which had a detrimental effect upon healing. However, Xelma treatment was considered successful in that it healed 80% of the wound before becoming static. Once the wound bed was sufficiently well granulated, a split skin graft was applied and graft take was 100%. A degree of tissue loss occurred after the skin graft, which was caused by the theatre dressing adhering to an area of the scalp which had already healed. After applying Xelma to this area of the scalp, the tissue healed once again.

The second study featured a 47-year-old male patient who had undergone insertion of metal pins to his ankle in 1996 due to a football injury. Following this, the patient experienced multiple problems which were unsuccessfully treated over

the course of 12 years, during which time his quality of life was adversely affected. In 2001 the patient underwent a skin graft which was a partial success, but complete healing was not achieved. From 2004 the patient was attended by different practitioners using a variety of treatment options, resulting in the layering of primary dressings, and the use of non-adherents, antimicrobials, odour controlling absorbents, and compression. None of these approaches resulted in a satisfactory outcome and the treatment protocols were not cost-effective. Strong, topical antimicrobial agents were being used to reduce the risk of infection and exudate levels and odour varied depending on the wound's bacterial levels.

In the summer of 2008, the patient was started on Xelma, the regimen followed was: 12 weeks of Xelma (weekly applications) with two weeks of rest, two weeks application (weekly applications) of Xelma, then four weeks without Xelma, and finally four weeks of Xelma treatment (applied weekly). In total, the Xelma treatment was 18 applications over a 24-week period. On 22 December 2008, the patient presented with a fully healed ulcer which remains healed to date. His routine now simply involves hydrating the healed skin with Epadem® cream (Mölnlycke Health Care).

The costs associated with treatment were as follows:

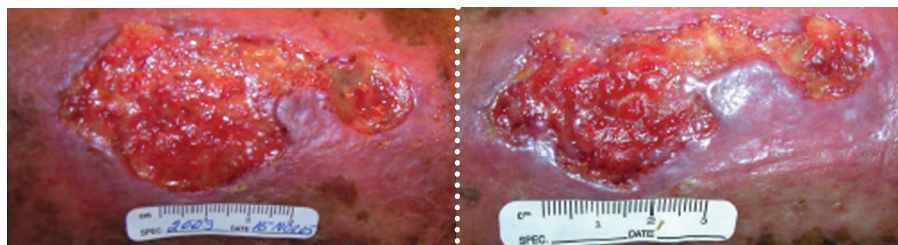
Costs = 18 syringes × £100 = £1,800
 Nursing time × £2 = £600
 (24 sessions) weekly
Total = £ 2,400

This compares to 12 years of previously unhealed leg ulceration based on an average of two dressings per week:

Nursing time: two sessions a week over 12 years × £50 = £31,500
 Dressings: 2 x a week for 12 years × £12 = £7,488
 Compression: Over 12 years × £20 = £12,480
Total = £51,168

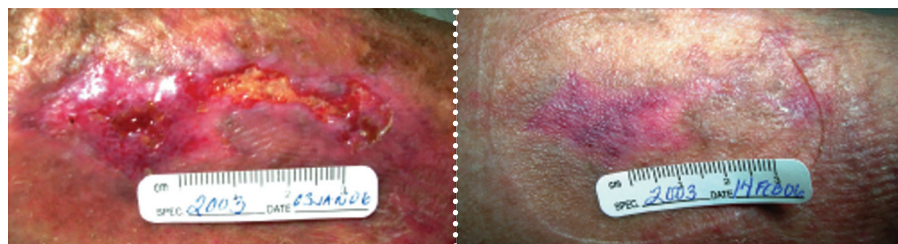
Costs of hospital admissions for skin grafts, outpatient appointments at vascular, dermatology and plastics were not taken into account, nor were the costs of other more expensive primary dressings considered. In the final analysis, it has taken a minimum of £51,000 to treat the patient without the wound ever healing. This is in stark contrast to the £2,400 costs required for healing with Xelma treatment, especially in view of the fact that the wound remains healed after five months of completion of treatment.

In a third study (Bond et al, 2009), a 70-year-old female with a long history of vascular problems dating from the age of 16 years presented in September 2006 with insect bites that had caused severe swelling and discolouration up to her knee, including damage to the site of a previous ulcer. The bites progressed to a wound that became ulcerated and developed into a chronic wound. When treated with compression therapy, the ulcer worsened leading to a 16-day hospital stay, during which time wound tissue and tendon were debrided and ulcer skin grafted. The patient was re-admitted in August 2007 for treatment of cellulitis. In June 2008, treatment was started with 1ml Xelma applied to the ulcer surface weekly. Mepitel was used as a secondary dressing. To date, around 40 applications of Xelma have been used at a cost of £3,500. Initially, the patient required pain management with opioids but with the application of Xelma the pain subsided almost immediately. This allowed opioid treatment to be titrated down and eventually removed. Largely due to pain reduction, the patient's quality of life increased as treatment



Photograph 1. VLU at beginning of run-in period (15 November, 2005). Large clean wound (17.9cm²).

Photograph 2. VLU at baseline (7 December, 2005). Still a large clean wound after run-in period.



Photograph 3. VLU at four weeks of treatment (5 January, 2006). Significant healing with re-epithelialisation and granulation tissue formation. No maceration and skin adjacent to wound appears healthy.

Photograph 4. VLU at 12 weeks of treatment (4 February, 2006). Qualitatively an excellently healed wound, with little scar tissue or contracture.

Figure 5. Healing progress of a patient treated with amlogenin over a 12-week period (Vowden et al, 2007a).

progressed and exudate levels and associated odour also subsided.

Other notable case studies

In an ongoing product evaluation with a view to allowing inclusion of Xelma into the formulary of the Wound Healing Unit at Bradford Royal Infirmary, Vowden et al (2007b) reported their experience of using the product on a wide variety of hard-to-heal wounds of mixed aetiology (17 wounds on 15 patients). Mean duration of wounds was 34 months and wounds comprised two rheumatoid ulcers, four wounds complicated by rheumatoid arthritis, five neuropathic foot ulcers, four venous ulcers, one mixed ulcer, and one pressure ulcer. Subsequently, an additional seven patients have been treated (three neuropathic diabetic foot ulcers and one neuroischaemic, two venous ulcers and one inflammatory ulcer).

In two patients treatment was discontinued due to infection and wound deterioration.

An early reduction in wound pain and exudate levels was reported in all cases. At the time of writing, only three of the

original 17 wounds remained unhealed. None of these three patients continues to receive amlogenin therapy. In the subsequent seven patients treated, six improved (four healed) and one had an episode of cellulitis but has subsequently improved (Vowden et al, 2007b).

In a case series involving nine participants with a total of 10 non-healing diabetic foot ulcers being treated with Xelma, McCardle et al (2009) demonstrated that only two of the 10 ulcers failed to improve following 12 weeks of treatment with Xelma. Eight of the ulcers improved, although the percentage of improvement varied widely (healing between 6–100% of the original ulcer area by study end). The authors emphasised the fact that those wounds that responded less dramatically during the 12-week treatment period appeared to continue healing following cessation of treatment with Xelma (McCardle et al, 2009). The authors concluded that Xelma was effective in the treatment of this series of static diabetic foot ulcers, successfully reducing the ulcerated area in eight out of ten cases where other treatments had failed.

In a small case study involving two patients with recalcitrant *pyoderma gangrenosum* of the lower leg lasting for an average of 11 months, Bertone et al (2007) reported that weekly application of amlogenin gel under occlusion for a period of four weeks improved the two lesions in terms of pain control, wound bed granulation, and wound size reduction after short-term therapy (Bertone et al, 2007).

In a study of two chronic wounds in patients with diabetes and in which the ulcers had been present for 18 months before the application of Xelma, Johnstone (2007) noted rapid improvement in granulation tissue formation compared with other treatments, the development of sensation in the neuropathic foot, reduced maceration during dressing changes, significant healing with healthy re-epithelialisation, and continued improvement after treatment with Xelma was discontinued. There were no adverse effects and levels of exudate had decreased during the use of Xelma compared with other dressing regimens (Johnstone 2007).

Economic evaluations

From a Markov model assessing the cost-effectiveness of using Xelma plus compression therapy versus compression therapy alone, in treating non-healing VLUs of more than six months' duration from the perspective of the NHS, Guest et al (2009) concluded that 60% of all wounds treated with Xelma plus compression therapy would be expected to heal within 12 months of the start of treatment, compared with 41% of wounds treated with compression therapy on its own ($p < 0.01$) (Figure 6).

In addition, 23% of all Xelma-treated wounds would be expected to improve compared with 18% of wounds in the compression therapy group. The difference in effectiveness between the groups is anticipated to lead to a 7% improvement in health gain among Xelma-treated participants when compared with those treated with compression therapy alone (0.800 versus 0.746 QALYs; $p < 0.01$) at 12

months after the start of treatment. Use of Xelma is also expected to result in a 10% reduction in NHS costs over 12 months from £4,261 (95% CI: £3,409; £5,114) to £3,816 (95% CI: £3,227; £4,405), due partly to a reduced need for nurse visits (Figure 7). As a result, Xelma plus compression therapy bandaging was found to be a dominant treatment. Treatment with Xelma is also expected to free-up NHS resources for other uses within the system.

Conclusion

Amelogenin, an ECM substitute, has been shown in a large trial with VLU to cause significant reductions in ulcer size, reduce levels of exudate, and reduce wound-associated pain. These findings are upheld by those made in smaller studies with both VLUs, diabetic foot ulcers (DFUs), and a variety of other hard-to-heal wounds of mixed aetiology. It is notable that wounds of greater than six months' duration and of an area exceeding 10cm² show the best response rates to treatment with amelogenin. **WUK**

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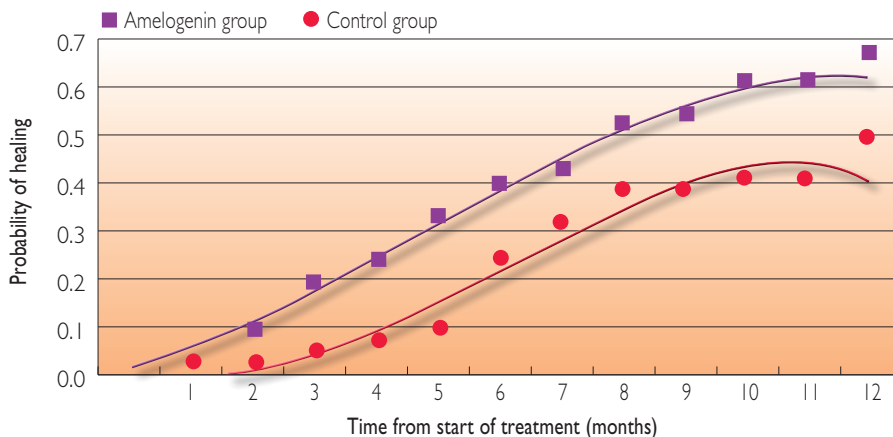


Figure 6. Expected probability of VLU healing over 12 months from the start of treatment (Guest et al, 2009).

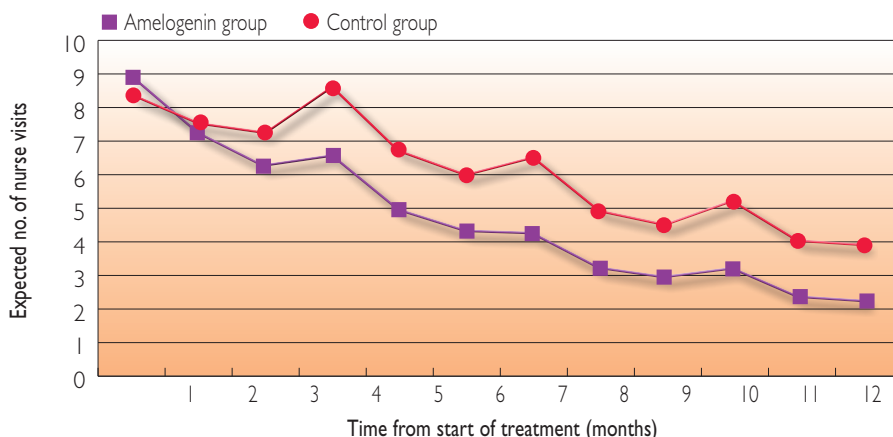


Figure 7. Expected number of nurse visits over 12 months from the start of treatment (Guest et al, 2009).

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Key points

- ▶▶ Hard-to-heal or chronic wounds are characterised by high levels of proteases, low levels of growth factors, and a compromised extracellular matrix (ECM).
- ▶▶ Xelma is an advanced treatment applied to the wound bed that behaves as a temporary or surrogate extracellular matrix.
- ▶▶ Xelma 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.