Pre-clinical Effectiveness of a Novel Biological Dressing for Chronic Wounds

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Background

Failure of wounds to heal may be due to a variety of factors. Where an imbalance in extracellular matrix degradation is implicated, it has shown that enhancement of the damaged underlying Extracellular Matrix (ECM) is clinically effective in promoting wound healing[1]. Several ECM enhancement dressings are available commercially and most are based on mammalian collagen preparations. However, the cost of these treatments is significant and they are often reserved for use only in the most complex chronic wounds. A lower cost alternative to mammalian derived ECM products is required for such products to be used widely. To address this unmet economic and medical need, Biovotec is developing a low cost ECM product based on eggshell membrane (ESM) - a by-product of commercial egg production.

In Eastern societies , ESM preparations have been used for many centuries to assist wound healing. More recently, ESM preparations have been shown to be effective as a dressing for skin graft donor sites[2]. In this study, ESM was harvested from domestic chicken eggs (*Gallus gallus)*, sterilized and applied essentially unmodified as overlapping membrane fragments. Results comparable to those of commercially available synthtic products including Biobrane™ were reported. The authors noted that the limited unit size of the ESM preparations would need to be overcome for its use to be potentially more extensive.

Here we present *in vitro* and *in vivo* wound healing data on an ESM preparation which has been incorprated into a thin dissolvable film for ease of application to wounds where enhancement of the wound bed ECM is indicated. The product is industrially scaleable and will have a distinct cost advantage to the end-user compared with currently marketed collagen and similar biologically derived products.

Methods

Purification of ESM and Manufacture of ESM Based Wound Dressing

Figure 1: Harvesting and purification of ESM fragments and manufacture of ESM powder. ESM is a fibrous material consisting of cysteine rich fibres which are densely packed and crosslinked. The processed powder may be incorporated into a variety of carriers to optimize the formulation for a range of wound types or may be administered to the wound directly as a powder.

Results

ESM inhibits MMP-2 and has an anti-inflammatory effect in vitro (Fig2). Both effects are inversely proportional to the size of ESM particles. Particles under 100µ have maximum effect.

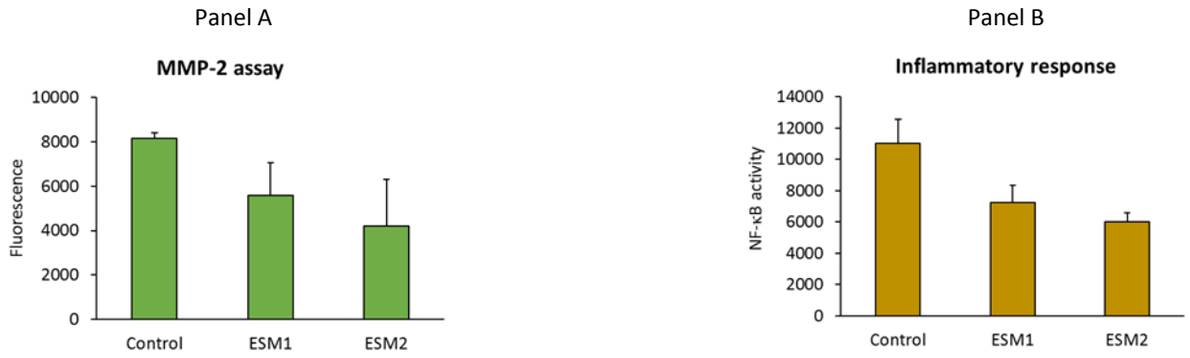
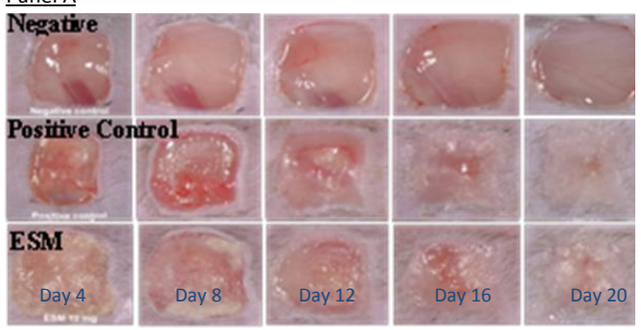


Fig 2: Two ESM preparations (ESM1 and ESM2) were assessed for protease inhibition and anti-inflammatory response. The preparations differed only in particle size. ESM1 had a mean particle size of greater than 100µ. ESM2 had a mean particle size of below 100µ. Panel A: The SensoLyte Generic MMP assay kit was used to detect the activity of recombinant MMP-2 where the fluorescence of the fluorophore substrate is proportional to protease activity. ESM reduces MMP-2 activity. Panel B: The anti-inflammatory action of ESM was studied in the U937 NF-κB -LUC cell system. After activation by bacterial LPS, a pro-inflammatory stimulus, endogenous NF-κB transcription factors bind to the DNA response elements and induce transcription of the luciferase reporter gene. The cells were incubated with the ESM fractions in presence of LPS to induce NF-κB activation. Luciferase was measured using the Bright-Glo Luciferase assay (Promega). ESM reduces the inflammatory response.

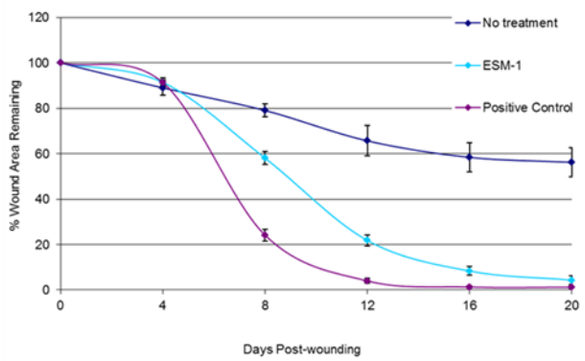
ESM stimulates wound healing in the db/db mouse model of delayed wound healing and is comparable to the positive control rhPDGF at day 20 post wounding (Fig 3).

Fig 3: This study compared the effectivensss of ESM powder with rhPDGF in the diabetic (db/db) mouse model (BKS.Cg-m Dock7^{m} +/+ Lepr^{db} /J). Full thickness 1cm^2 wounds were made dorsally (1 per animal; n=10 per group) and covered with a transparent wound dressing film. The ESM group was treated twice at Day 0 & Day 4 where as rhPDGF was administered every day from day 0 to day 6. Observations and wound size measurements were made at Days 4, 8, 12, 16 and 20 (Panel A) and % wound area remaining calculated (Panel B).

Panel A



Panel B



Conclusion

ESM is a fibrous protein matrix derived from an animal extracellular matrix source and food industry by-product. It is:

- A collagen-like protein with additional extracellular matrix components
- An inhibitor major MMP's implicated in delayed wound healing & is anti-inflammatory
- Inducer of wound healing through connective tissue formation and angiogenesis

ESM presents significant unit cost benefits over collagen for manufacture of biologic wound based dressings and could potentially expand the use of biological dressings to all wounds at risk of delayed wound healing.

References

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