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Eggshell Membrane – An Equivalent of Extracellular Matrix (ECM) in Avian Egg has Modulating Wound Healing Properties

Tram T. Vuong[1], Sissel B. Rønning[1], Henri Pierre[2], Enda Kenny[2], Ralf Schmidt[2], Jeff Hart[3], Andrea Bell[3], Kristian Prydz[4] and Mona E. Pedersen[1]

[1]Nofima AS, Oslosveien 1, 1430 Ås, Norway; [2]Biovotec AS, Hoffsveien 21-23, 0275 Oslo, Norway; [3]Cica Biomedical Ltd, Innovation Centre, UK; [4]Department of Biosciences, University of, Norway.
Contact Email: tram.thu.vuong@nofima.no

Background

Components of the extracellular matrix (ECM) play an important role in normal wound healing and the unregulated degradation of ECM components impairs healing. This has led to the development of new therapies that aim to reduce the degradation of ECM or to re-establish undamaged ECM.

In Eastern societies, eggshell membrane (ESM) preparations have been used for centuries to assist healing of burn and cutaneous wounds. More recently, ESM preparations have been shown to be effective as a dressing for skin graft donor sites[1]. Biovotec AS, a biotech company, is developing a novel, low cost wound healing product based on the raw material of ESM. There are currently limited studies on ESM mechanisms. In this study, we characterized the composition and investigated the effect of ESM in cell models mimicking the wound healing processes. In addition, a pre-clinical study was performed in diabetic db/db mouse.

Results

ESM is a fibrous protein matrix containing collagen and GAGs

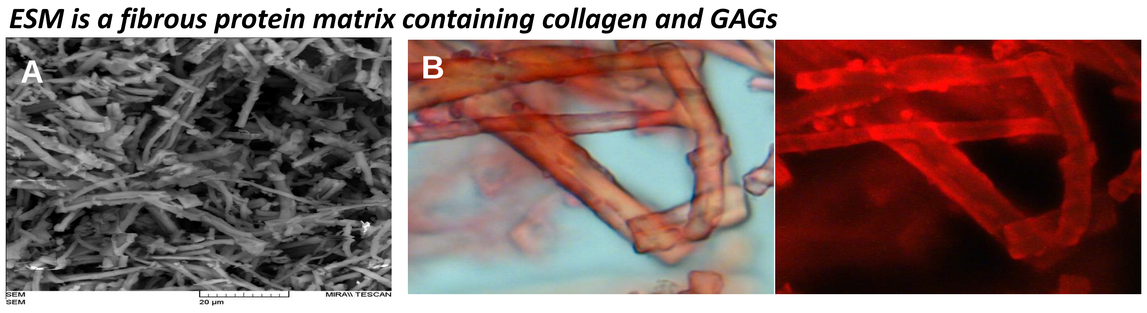

Figure 1. *A) SEM photograph of ESM powder showing fibrous protein, collagen-like structure. B) Staining with Sirius Red demonstrates the presence collagens (left panel) and the crosslinking of Col I and III at surface of fibers (right panel).*

Table 1. *The composition of GAG types in ESM*

Disaccharides	% record
HA	81
CS-0	6
CS-4	12
CS-6	1
KS	ND
HS	ND

HA=hyaluronic acid, CS=chondroitin sulphate, KS=keratin sulphate, HS=heparin sulphate

ESM has anti-inflammatory effect and inhibits MMP-2 and in vitro

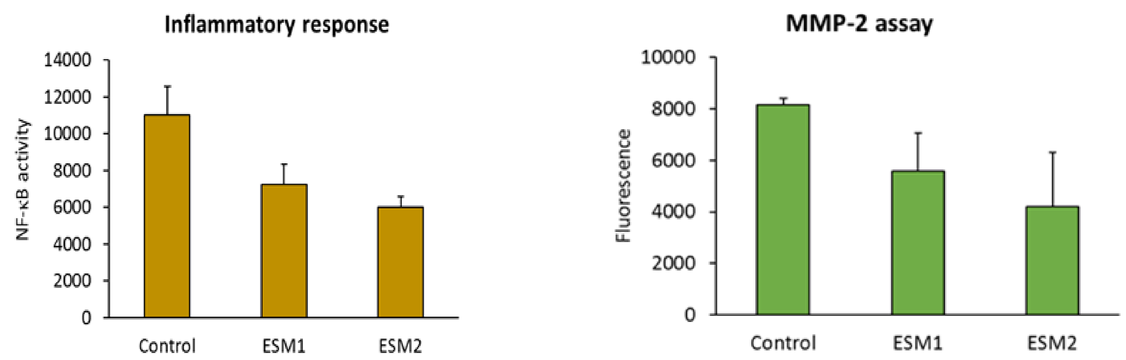

Figure 2. *A) The anti-inflammatory action of ESM was studied in the U937 NF-κB -LUC cell system. After activation by bacterial LPS, a pro-inflammatory stimulus, endogenous NF-κB transcription factors bind to the DNA response elements and induce transcription of the luciferase reporter gene. The cells were incubated with the ESM fractions in presence of LPS and luciferase was measured using the Bright-Glo Luciferase assay (Promega). ESM reduces the pro-inflammatory response. B) 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 relative to the untreated control. Similar result is observed for MMP-9 (data not shown). ESM1= particle size >100 µm and ESM2 = particle size <100 µm.*

ESM regulates secretion of anti-inflammatory cytokines in immune cells

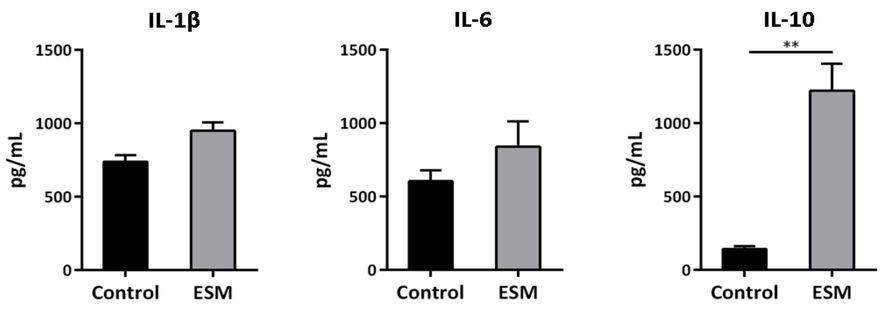

Figure 3. *An inflammatory response was triggered in differentiated macrophage-like THP-1 cells by bacterial LPS. ESM was added to the cells and the concentration of secreted pro- (IL-1β and IL-6) and anti-inflammatory (IL-10) cytokines were assessed by ELISA. ESM strongly induces the production and secretion of the anti-inflammatory IL-10, a key regulator of the inflammatory response.*

ESM stimulates fibroblast-to-myofibroblast transition of human dermal primary fibroblasts

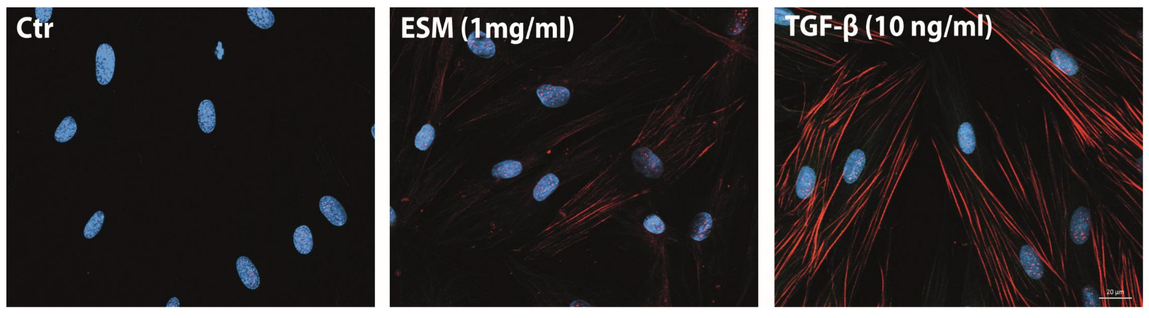

Figure 4. *The fibroblast-to-myofibroblast differentiation was investigated by the myofibroblast marker α-SMA. Fibroblasts were incubated with ESM or TGF-β, a known myofibroblast inducer, for 2 days. The expression of α-SMA was assessed by immunofluorescence. ESM induces fibroblasts to express the myofibroblast marker in similar to TGF-β.*

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

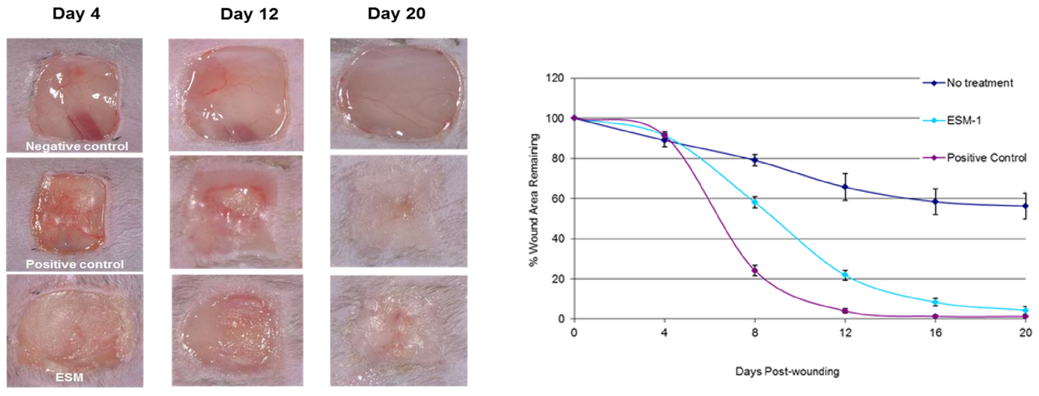

Figure 5. *A) This study compared the effectiveness of ESM powder with rh-PDGF-BB/rh-TGF-α in the diabetic (db/db) mouse model (BKS.Cg-m Dock7^{m} +/+ Lepr^{db}/J). Full thickness 1cm^2 wounds were made dorsally (1 per animal; n=10 per group) and covered with a transparent wound dressing film. The ESM group was treated twice at Day 0 & Day 4 where as rh-PDGF-BB/rh-TGF-α was administered every day from day 0 to day 6. Observations and wound size measurements were made at Days 4, 12 and 20 (A) and % wound area remaining calculated (B).*

Conclusion

ESM presents properties of promoting wound healing and may potentially become a novel biological wound dressing that can be used for normal wounds and those at risk of delayed healing.

This is supported by multi properties of ESM:

- ✓ Contains collagens and GAGs
- ✓ An inhibitor of major MMP's implicated in delayed wound healing
- ✓ Has anti-inflammatory property
- ✓ An inducer of myofibroblast differentiation
- ✓ Promotes wound healing in pre-clinical trials

Materials

Eggshell membrane (ESM) was washed and activated before milling to powder at Biovotec AS. The mean particle size of ESM is <100 µm.

References

[1] Yang, J-Y; Chuang, S-S; Yang, W-G and Tsay, P-K. Chang Gung Med J. 2003; 26: 253-159.

***Nofima AS**, <Fyll inn din arbeidsstedsadresse>, Tel: +47 77 62 90 00, www.nofima.no, post@nofima.no - Norwegian Institute of Food, Fisheries and Aquaculture Research*

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