Development of a wound healing assay using inclusion bodies

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The regeneration capacity of adult human skin is limited, resulting frequently in scarring. In addition, chronic wounds are a serious issue in clinical settings. Therefore, improving the regeneration capacity of skin is an important goal in clinical research. The epidermal lipoxigenase ALOXe3 in mammals is involved in the regulation of keratinocyte differentiation. In the Mexican salamander (axolotl) an epidermal lipoxigenase (AmbLOXe) could be detected during limb redevelopment. Studies have shown that human cells also respond to the epidermal lipoxigenase from the axolotl, thus migrating and closing wounds faster with AmbLOXe. Hence, epidermal lipoxigenases offer a promising tool for clinical applications. To study the effects of these enzymes in wound healing, they were expressed in epidermal lipoxygenase from the axolotl, thus migrating and closing wounds faster with AmbLOXe. Hence, epidermal lipoxigenases offer a promising tool for clinical applications. To study the effects of these enzymes in wound healing, they were expressed in epidermal lipoxygenase from the axolotl, thus migrating and closing wounds faster with AmbLOXe. Hence, epidermal lipoxigenases offer a promising tool for clinical applications. To study the effects of these enzymes in wound healing, they were expressed in Escherichia coli. Both lipoxigenases could be produced only in insoluble protein aggregates (inclusion bodies (IBs)). However, the production of IBs is not necessarily disadvantageous. Aggregated enzymes may show activity and display a greater robustness towards mechanical stress.

Production and purification of lipoxigenase IBs:

Inclusion bodies (IBs):
- aggregation of non-folded, partially folded and correctly folded protein (reservoir for functional enzyme)
- mechanically stable
- size: 50 nm – 1 μm

Results

Figure 1: Concentration determination of ALOXe3 and AmbLOXe using densitometry yield of inclusion bodies (IBs)
- ALOXe3 (83 kDa):
  - 175 mg in 1 L culture broth
- AmbLOXe (74 kDa):
  - 171 mg in 1 L culture broth

Figure 2: Possible cell injury methods used in wound healing assays; modified from [3]; red squares indicate methods used in combination with inclusion bodies for this study

(A) scratch assay (results not shown),
(B) stamp assay,
(C) thermal wounding,
(D) electrical wounding using Electric Cell-Substrate Impedance Sensing (ECIS) (results not shown),
(E) optical wounding using laser,
(F) culture inserts (results presented on this poster)

Figure 3: Measurement of wound areas using MRI wound healing tool macro for ImageJ

(A) after removing cell culture insert; t = 0 h
(B) after wound healing; t = 12 h
(1) Control (no IBs)
(2) Coating with GFP IBs
(3) Coating with ALOXe3 IBs
(4) Coating with AmbLOXe IBs

Figure 4: Results of wound healing assay with culture inserts
Coating with IBs & direct addition of IBs to culture media (62.5 mg/cm²)
Control (no IBs)
GFP IBs (negative control)
ALOXe3 IBs
AmbLOXe IBs

Conclusion and outlook

- Inclusion bodies (IBs) of epidermal lipoxigenases could be successfully produced in E. coli, have been purified from bacterial cell culture and were tested for sterility in animal cell culture.
- IBs were adapted for testing in in vitro wound healing assays by coating cell culture surfaces.
- Using ECIS and culture inserts for wounding led to reproducible wound areas, and thus improved repeatability of experiments.
- Coating of cell culture surfaces with AmbLOXe IBs as well as direct addition of AmbLOXe IBs to media resulted in a faster migration of cells, thus closing wounds faster. Similar results could be obtained for ALOXe3 (Figure 3 and 4).
- Both lipoxigenases (AmbLOXe and ALOXe3) seem to promote wound closure and are promising candidates for clinical applications, but the exact mechanism of action remains to be elucidated.

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