Developing a diagnostic assay for skin burn depth using methylated DNA in blister fluid.

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In Australia, approximately 5,700 people are hospitalised annually due to burns of the skin, and it initially costs $71,000 on average to treat a burns patient – thus posing a burden on the Australian health care system[1]. One major clinical problem is determining the depth of the burn, as this depth is a good indicator of burn healing outcome, and also guides a clinician's decision whether to use aggressive burn treatment strategies such as skin grafts[2]. **However, current diagnostic techniques for measuring burn depth are hard to interpret, expensive and time consuming[3].**

Aim and hypothesis

This project will address the problem of accurately and quickly measuring burn depth, by developing a proof-of-principal diagnostic assay using biomolecules (methylated DNA) in blister fluid – a fluid released from skin burns. To the best of our knowledge, this will be the first study to measure methylated DNA in blister fluid, addressing a major scientific knowledge gap. We hypothesise that our DNA methylation assay which is designed to detect damage specific to the deep layer of the skin (dermis), will have an elevated signal in a deep burn compared to a superficial burn.

Key results

1) Does DNA exist in blister fluid and how much DNA? Basic profiling of DNA in blister fluid.

Blister fluid has a similar composition to plasma, so we extracted the DNA from blister fluid using the QIAamp MinElute ccfDNA kit (QIAGEN). Subsequently, we quantified the extracted DNA using Qubit and ran this DNA on a 2% E-Gel (ThermoFisher), the results shown below in Figure 1.

- DNA yield is approximately 1.5 µg / mL of blister fluid.
- Conservatively, our current assay could diagnose burn depth using 100uL-150uL of blister fluid (45 ng / reaction).
- In future versions of our assay, it may be possible to only require 50uL of blister fluid, due to multiplexing.

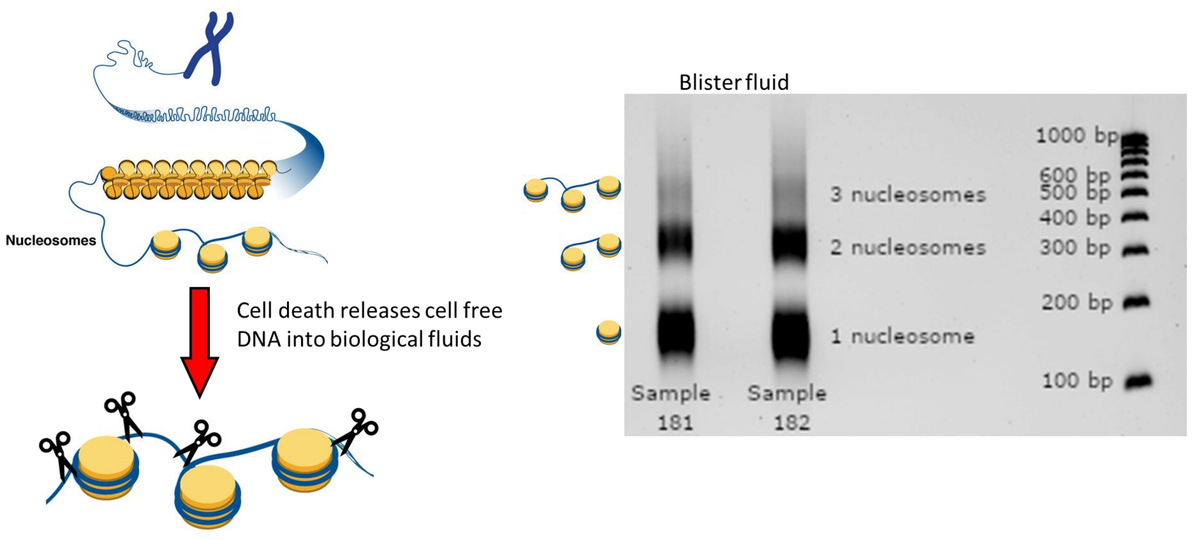

Figure 1: Gel electrophoresis of DNA extracted from blister fluid (Sample 181 and 182). The extracted DNA shows the typical banding profile observed in plasma cell free DNA. Each band on the gel shows that DNA size increases in 150 base pair (bp) increments, which is approximately the length of DNA wrapped around a nucleosome, protecting the DNA from enzymatic degradation.

2) Whole genome bisulfite sequencing, assay development and screening.

We performed the first whole genome bisulfite sequencing on blister fluid, which gave us information on the methylation pattern of the entire blister fluid genome. Using this information and public databases we developed two skin damage assays; **1)** Dermis assay detecting skin damage in the deep (dermal layer) of the skin, and **2)** Epidermis assay detecting skin damage to the superficial (epidermal layer) of the skin. Shown below are the details of assay development and screening process.

Dermis damage DNA methylation assay
• 9 genomic regions screened.
• 71 primer pairs designed and screened.
• 7 TaqMan probes designed and screened.
• One final primer / probe assay.

Epidermis damage DNA methylation assay
• 8 genomic regions screened.
• 113 primer pairs designed and screened.
• 2 TaqMan probes designed and screened.
• One final primer / probe assay.

Bioinformatics to find candidates
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qPCR Methylated vs Unmethylated control screen
↓
qPCR Tissue screen (Dermis, Epidermis, Blood, Skin)
↓
qPCR Primer iteration (add / subtract bases from 5' primers)
↓
qPCR Tissue screen 2 (Dermis, Epidermis, Blood, Skin) SYBR / TaqMan Probe.
↓
Final dermis / epidermis specific skin damage DNA methylation assay.

3) Final assay validation on blister fluid samples using digital PCR (QIAcuity).

We validated our dermal / epidermal tissue damage assay on blister fluid using digital PCR. We found that our dermal tissue damage assay showed an elevated signal for a deep burn, compared to a superficial burn. In addition, our epidermal tissue damage assay showed similar expression between burn types. There is some background signal for our dermal tissue damage assay.

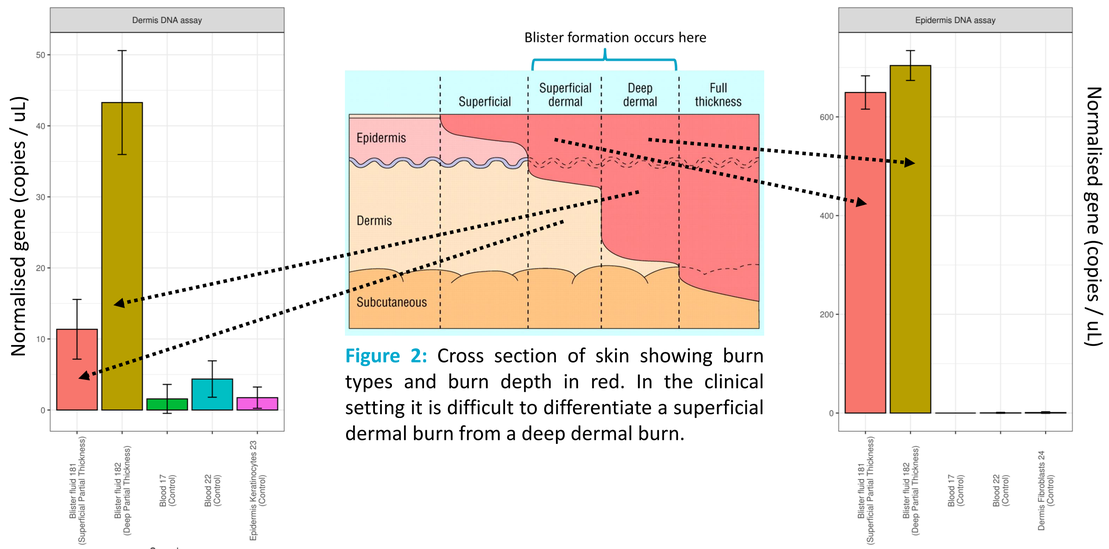

Figure 2: Cross section of skin showing burn types and burn depth in red. In the clinical setting it is difficult to differentiate a superficial dermal burn from a deep dermal burn.

Conclusion

We are the first to develop a DNA methylation assay for burn depth using blister fluid, and have demonstrated its utility to differentiate a deep burn from a superficial burn. Our assay can help guide the clinicians burn treatment strategy, ultimately benefitting the patient and their quality of life.

FOR FURTHER INFORMATION

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