Coherency Image Analysis to Quantify Collagen Architecture: Implications in Scar Assessment

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Supporting Information

Table S1 – Current quantitative methods to determine collagen architecture in the skin

| Method                                           | Reference paper | Summary of Key Findings                                                                                     |
|--------------------------------------------------|-----------------|-------------------------------------------------------------------------------------------------------------|
| Orientation/ Analysis (ImageJ)                    |                 | • Successfully differentiates between normal and scar modelled environments in vitro                        |
|                                                  |                 | • Greater sensitivity when compared to Fourier analysis methods                                            |
|                                                  |                 | • Suitable for analysing coherency differences in the collagen deposited by human keloid scars             |
|                                                  |                 | • Quick and easy quantitative analysis method of assessing collagen structure in scarring                 |
| Fourier Analysis and Collagen Orientation Index (COI) | Van Zuijlen et al. 1 Verhaegen et al. 2 | • Study compared the accuracy of Fourier analysis of confocal images with individual observers of polarised light and the confocal images of scar tissue and normal skin. 1 |
|                                                  |                 | • In follow up work they used the COI to try and differentiate between normal skin, normotrophic, hypertrophic, and keloid scars. 2 |
|                                                  |                 | • Fourier analysis was able to achieve a superior measurement of collagen orientation compared with subjective histological evaluation by several experts in the field. 1 |
|                                                  |                 | • The COI (based on Fourier analysis) was significantly less for normal skin when compared to scar but was unable to define differences between the scar types. 2 |
| Second Harmonic Generation (SHG) imaging          | Tanaka et al. 3  | • In vivo SHG imaging of dermal collagen fibres following burns in a rat model. 3 |
|                                                  |                 | • Similar to ex vivo analysis of skin sections, SHG imaging is able to discriminate between the effects of thermal denaturation of collagen molecules following a burn injury. 3 |
|                                                  |                 | • Expensive specialised equipment required.                                                                |
| Confocal Microscopy                               | Khorasani et al. 4 | • Scar collagen morphology comparing differences in full thickness burns and normal tissue using fractal dimension and lacunarity analysis was achieved. 4 |
|                                                  |                 | • Confirmed with transmission electron microscopy for comparison. 4                                         |
|                                                  |                 | • More sensitive than Fourier analysis for quantification of scar morphology.                              |
| Histological Staining (e.g. Masson’s trichrome or Herovici) | Rawlins et al. 5 Sanders et al. 6 | • Able to determine the differences in mature burn scars with normal skin.  |
|                                                  |                 | • Herovici staining can differentiate type I collagen (red) from type III collagen (blue). 5                 |
|                                                  |                 | • Masson’s suitable for measuring differences’ in collagen density in mechanically stressed vs normal skin with computer aided image processing. 6 |
|                                                  |                 | • Quantification of collagen possible with post image analysis software of pixel colour thresholding.       |
|                                                  |                 | • Unable to be used in vitro.                                                                             |
Figure S1 – Inter and intra-rater reliability of the coherency measurement for *in vitro* collagen deposition in a scar like environment (A) and skin tissue sections (B). No significant difference was observed between rater 1’s repeated measures or between rater 1 and rater 2 for the *in vitro* (n=18 images) or the *in vivo* samples (n=50 images). Data displayed as mean ± SD and statistically assessed with a one-way ANOVA followed by a Bonferroni comparison test (p<0.05).

Supporting Information References

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