MEDICAL UNIVERSITY OF VIENNA

Monitoring of Cutaneous Wound Healing in Diabetic and Non-Diabetic Mice using Optical Coherence Tomography Angiography

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Background and aim

- **Diabetes mellitus**, a chronic metabolic disorder, is a major cause for vascular diseases and is also associated with impaired wound healing.
- **Optical Coherence Tomography Angiography** (OCTA) is a non-invasive imaging modality that can visualize the superficial vasculature in skin, providing a unique window into the condition of the vascular network in vivo.
- **Aim**: To monitor the changes to the vascular network during wound healing in healthy and diabetic mice using OCTA.

Methods

Wound healing model

- Excision in mouse ear with 1 mm diameter
- Wound splint to minimize tissue contraction
- Healthy (C57BL/6) and diabetic (db/db) mice
- 4 groups: chitosan hydrogel, fibrin + VEGF + PDGF, fibrin, neg. control
- 5-6 animals per group
- Ketamine xylacine anaesthesia / isofluorane anesthesia

OCTA imaging

- Custom-built swept source OCT system at 1300 nm
- Lateral resolution ~13 µm, depth of focus ~200 µm
- Split-spectrum amplitude decorrelation angiography
- 4.86 mm x 4.86 mm
- Images taken of baseline and 0, 1, 4, 7, 12 and 18 days after wounding

Vascular analysis

- Sato filter of curvilinear structures
- Contrast limited adaptive histogram equalization
- Thresholding using Otsu's method

Vessel parameters

- Vessel density
- Normalized vessel length
- Bifurcations per vessel length
- Vessel tortuosity

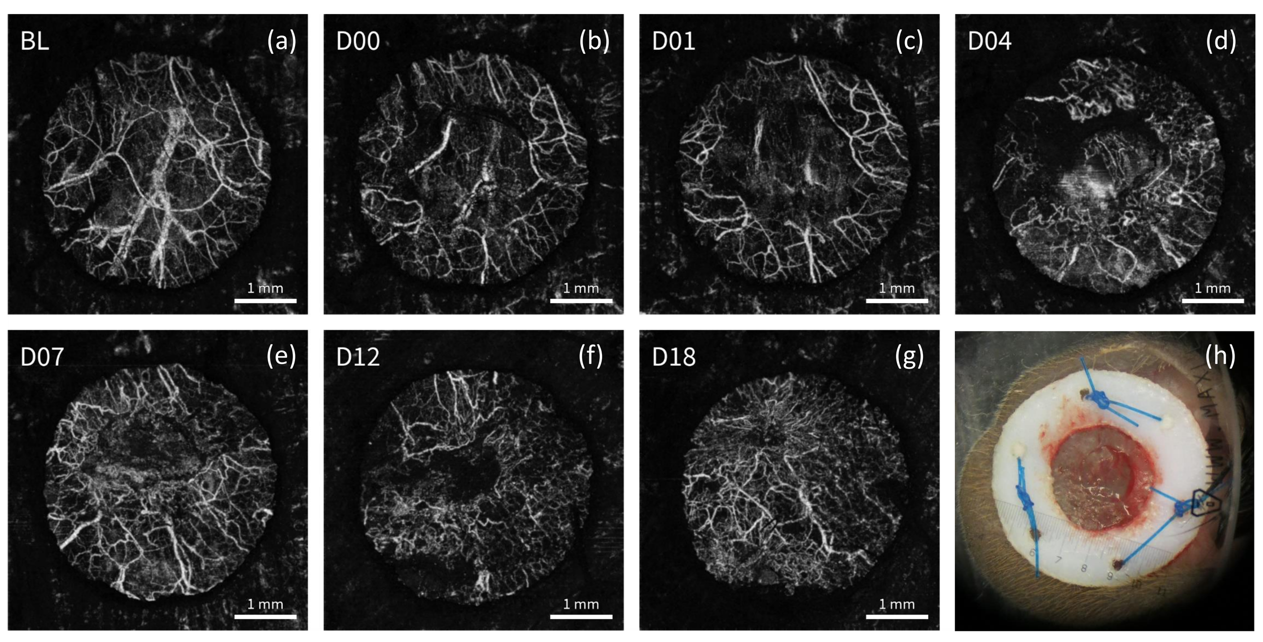

Figure 1: (a) – (g): OCTA images of dermal wound healing. Non-diabetic mouse, no treatment control. (h): Wound splinting in the mouse ear pinna wound model. [1]

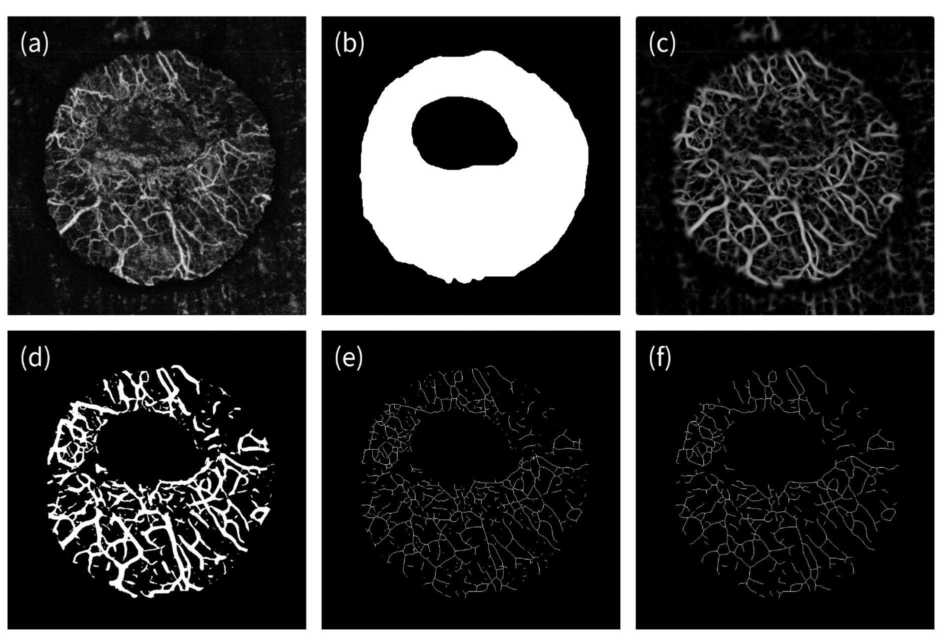

Figure 3: Steps during vessel analysis. [1]
(a) Original angiogram
(b) Region of interest
(c) Sato filter
(d) Thresholding
(e) Skeletonization
(f) Removal of short vessel fragments

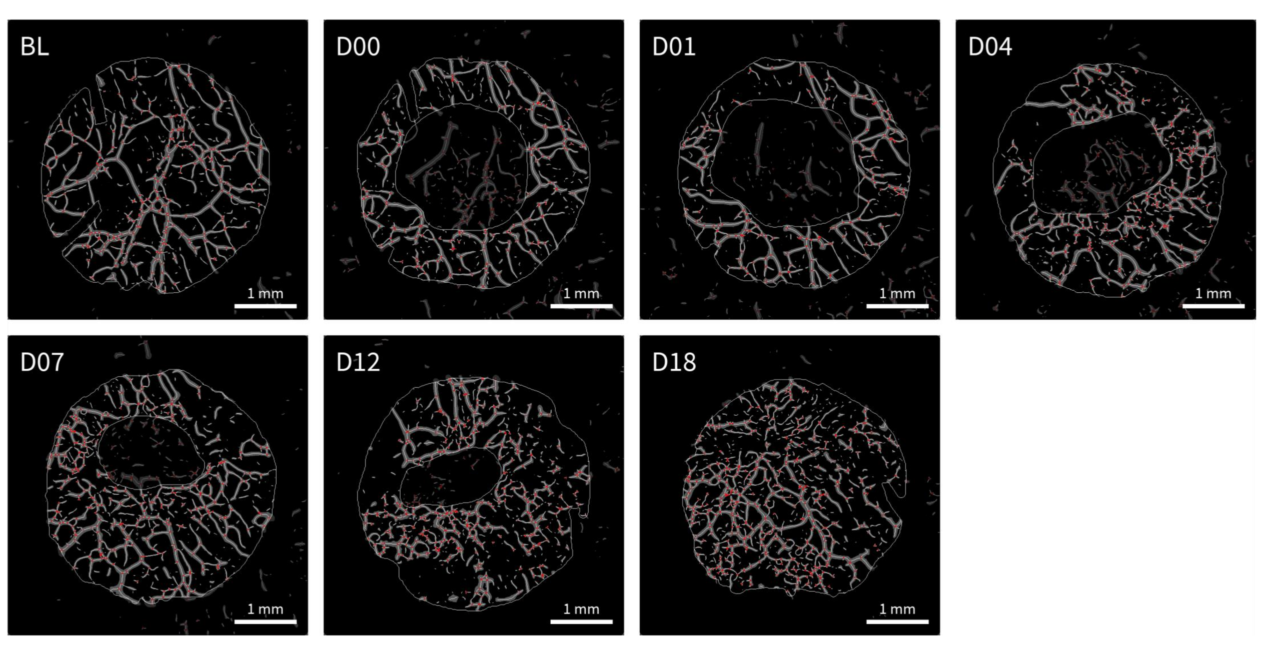

Figure 4: Vascular network derived from OCT angiograms. [1]

Results

- Wound healing phases can be identified: Hemostasis, inflammation, proliferation, and remodeling.
- Vessel density, vessel length, number of bifurcations and vessel tortuosity reached peak values 28-47 %, 39-52 %, 33-48 %, and 3-8 % above baseline, respectively, on study days 4 to 7 (proliferative phase).
- Values decreased afterwards but still elevated on day 18 (remodeling phase).

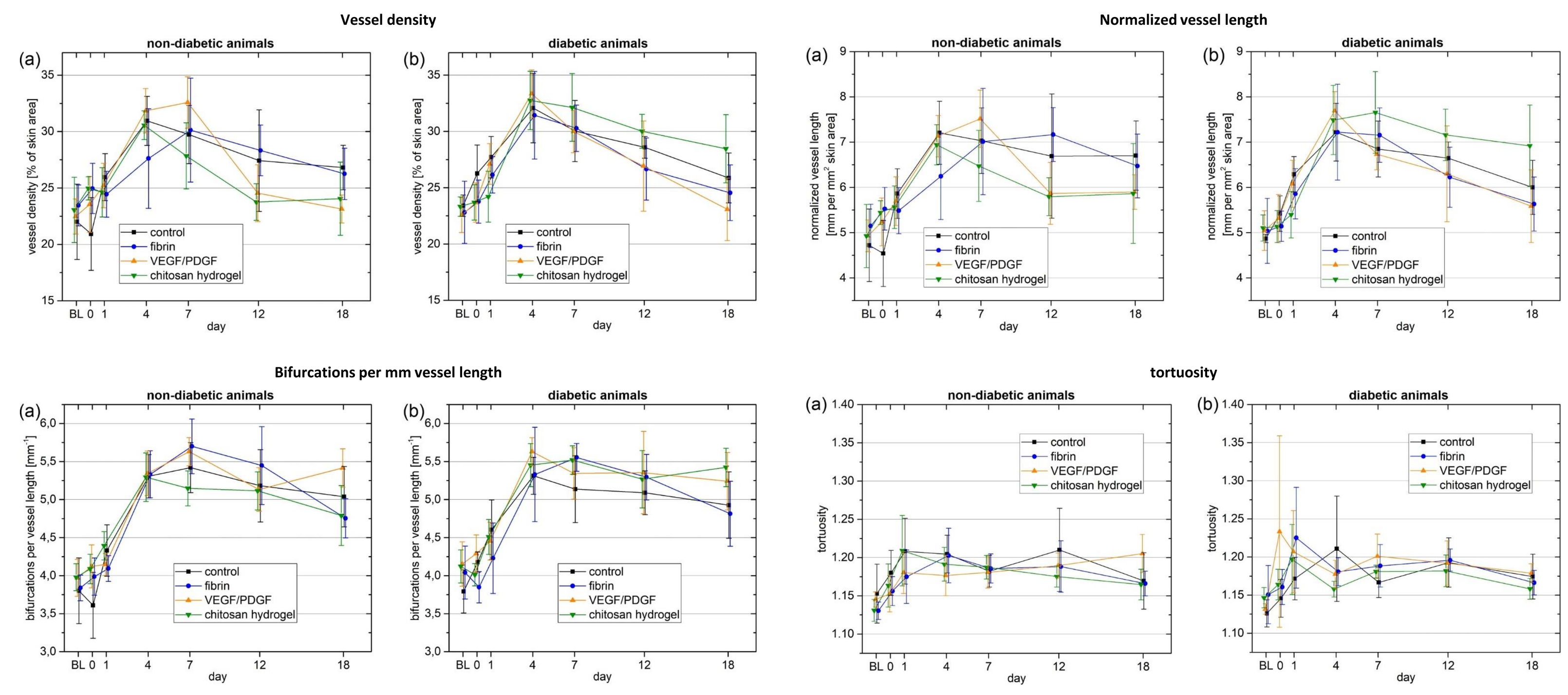

Figure 5: Vascular parameters derived from OCT angiograms. [1]

Follow-up study (in progress)

- No wound splint
- 8-9 animals per group
- MMFK anesthesia
- Convolutional neural network (CNN) vessel segmentation
- Vessel tortuosity: $\frac{\textit{Path length along vessel}}{\textit{Length of a straight line}}$
  - Previously: Calculated per vessel segment; weighted average over all vessel segments.
  - Improved: Calculated per fixed vessel length of 20 px; averaged without weighting. (Longer vessels will contain more 20 px sections.)

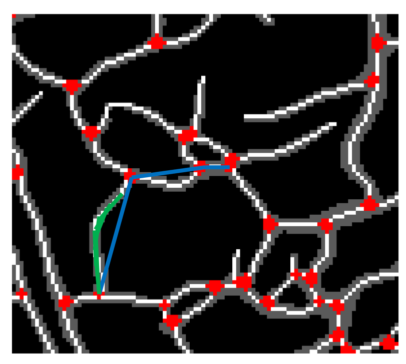

Figure 6: Illustration of improved vessel tortuosity evaluation.

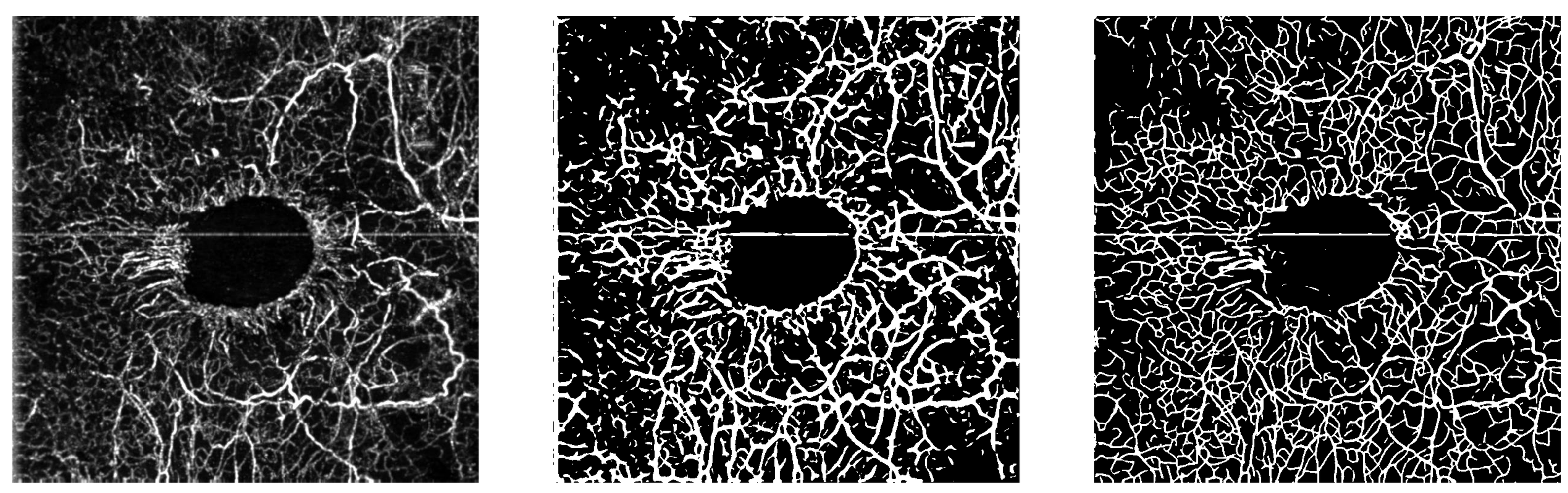

Figure 7: New segmentation. Left: OCT angiogram. Center: Previous segmentation. Right: CNN segmentation.

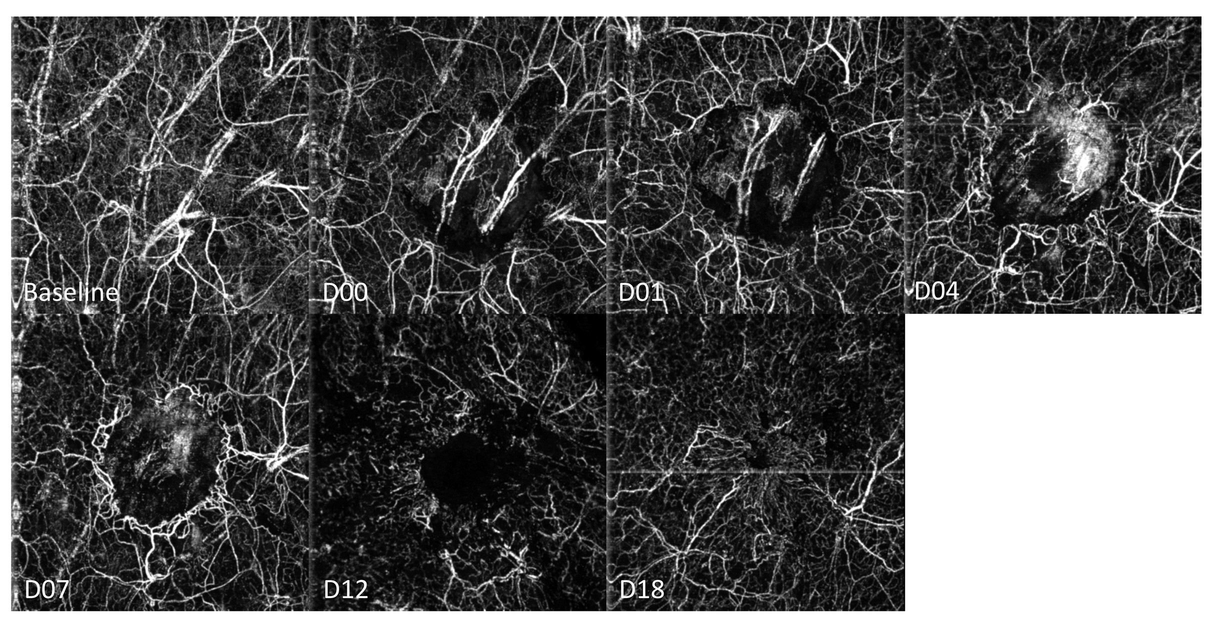

Figure 8: OCTA images of dermal wound healing. Non-diabetic mouse, no treatment control. Follow-up study.

Conclusion

OCTA can be used for in vivo monitoring of the dynamic changes in the vascular network during wound healing and may serve as a valuable tool in preclinical research studying impaired vascular remodeling and potential treatment strategies.

References

[1]: Pfister, M.; Schützenberger, K.; Schäfer, B.J.; Puchner, S.; Stegmann, H.; Hohenadl, C.; Mildner, M.; Garhöfer, G.; Schmetterer, L.; Werkmeister, R.M. Optical Coherence Tomography Angiography Monitors Cutaneous Wound Healing under Angiogenesis-Promoting Treatment in Diabetic and Non-Diabetic Mice. Appl. Sci. 2021, 11, 2447. https://doi.org/10.3390/app11052447