Supplementary Methods

S1. Statistical Analysis

S1.1. Generalized linear mixed models

The objective of the generalized linear mixed models was to examine differences in mean features across the groups and individual time points. To accomplish this objective while accounting for the non-normal distribution of the features, PROC GLIMMIX or generalized linear models with repeated statement (PROC GENMOD) and an appropriate link function were used. Model fit was determined using the ratio of chi square to its degrees of freedom \( \chi^2 / df \), where a ratio larger than 2 indicates an inadequate fit (32). After fitting the model, pairwise comparisons were conducted using the LSMESTIMATE statement between the individual groups and pairs of groups and the p-values were adjusted for multiple comparisons using the Sidak test (33). A random subject (mouse) effect nested within group was included and time was treated as a classification (categorical) variable to determine whether the treatment effect varies at one or more specific time points. The models were adjusted for time, weight at each time point, treatment group (4 groups using one as a reference), and the treatment group x time interaction. Features that demonstrated interactions that were significant \( p < 0.05 \) or approaching significance \( p < 0.1 \) at one or more individual time points for at least one pairwise comparison of treatment groups were selected for inclusion in the next analysis phase (path analysis using latent variables).

S1.2. Path analysis using latent variables

The objective of path analysis was two-fold: i) to understand the associations among the features when grouped into a higher level reflecting the wound healing components of angiogenic, structural, and metabolic features; ii) to use one model that incorporates all the wound healing components while accounting for trends over time. Time points were collapsed (combined) to correspond to the temporal stages of the wound healing process. Days 1 and 3 were combined to indicate the inflammatory stage; Days 7, 10, and 14 for the proliferative stage; and Days 21 and 28 for the remodeling stage. Day 0 was kept separate to indicate the pre-wound time. This approach was beneficial because the features had a significant interaction with time at some but not all of the time points in the mixed models, and so collapsing features across time points...
increased the analytic sample size, reduced the number of parameters to estimate, and simplified interpretation of model findings.

The modeling process was conducted over 2 steps, consisting of the creation of latent factor scores followed by path analysis, according to the method of Ines Devlieger and Yves Rosseel (34). This modeling process was chosen as an alternative to structural equation modeling (SEM) due to the relatively small sample size; it yields regression parameters similar to SEM, and handles misspecification better than SEM due to the stepwise process.

**Step 1: Creation of the latent factor scores**

Within each mouse treatment group, three latent variables were created to represent each wound healing component using the FACTOR procedure in SAS (factor analysis). Factor analysis was chosen instead of principal components because it assumes that there is error variance, which is more realistic in this context since we are trying to measure the underlying constructs of the wound healing components that are subject to measurement error. No rotation was needed since only one factor was created for each wound healing component. The prior communality estimates (estimate of the variance of an item that is shared with other items) were specified as MAX (largest absolute correlation of a feature with any other feature). To account for the trends over time, each latent variable was created using the corresponding features from Day 0 and the collapsed time points (Days 1 and 3 combined; Days 7, 10 and 14 combined; Days 21 and 28 combined). The respective features used to create the three latent variables and the wound healing components they represent were: OCTA_MI over Days 0, 1 and 3 combined, Days 7, 10, and 14 combined, Days 21 and 28 combined (latent variable representing the wound healing component of angiogenesis over time), SHG_A (structural integrity), and FLIM_L, FLIM_NADH_I, FLIM_Bound_NADH_F (metabolism). Each latent variable was then scored to create a latent factor score, by applying the coefficients of the latent variables to the raw data using the SCORE procedure in SAS. The treatment group-specific variances and covariances of the latent factor scores were estimated using the CORR procedure by specifying the options ‘cov nocorr outp=cov(type=cov)’ in the CORR statement. The mouse weight at Day 0 was incorporated in the variance-covariance matrix estimation for adjustment purposes.

**Step 2: Path analysis**
Each treatment group-specific variance and covariance matrix of the latent factor scores was entered into a path analysis using the CALIS procedure in SAS. The relationships between the three latent factors (angiogenesis, structural integrity, metabolism) were tested by specifying a direct path (coefficient) between each latent factor and the other two. For example, a path was specified from angiogenesis to structural integrity and metabolism to test whether angiogenesis has an effect on structural integrity and/or metabolism in that direction. For each path entry, the direct path from the latent factor to one of the other two was fixed to 1 to facilitate interpretation. One path analysis was tested for each mouse treatment group, leading to four path analyses. Standardized path coefficients are reported to enable comparison of path effects across mouse treatment groups. Model fit was assessed using Standardized Root Mean Square Residual (SRMSR, < 0.08) and Bentler’s Comparative Fit Index (CFI, > 0.95). Significance was determined using the absolute value of the t-statistic (t-value > 1.96 is statistically significant at p < 0.05).

**Supplementary Figures**

![Image](image_url)

*Figure S1. Illustration of the location (white square) where the NAD(P)H and FAD images were taken with respect to the wound (white dotted circle).*
Figure S2. Mouse skin mounting for imaging. A) A cover glass was placed on the back of the mouse with the wound on the center. B) The mouse was gently placed on the XY stage with the anesthesia nose cone.

Figure S3. Representative photographs of wounds from mice from each group, at each imaging day.
Figure S4. Representative SHG images of wounds from mice from each group, at each imaging day. The green signal shows the collagen fibers and the red signal shows hair follicles and scab.

Figure S5. Representative OCTA images of wounds from mice from each group, at each imaging day.
Figure S6. Difference in cell size from Day 0 (A) to Day 3 (B). Cells on Day 3 present larger size and sharper nuclei borders.

Figure S7. Representative NAD(P)H FLIM images of an area near the wounds of mice from each group, at each imaging day.
Figure S8. FAD images showing epithelial cell re-growth inside the wound at Days 10 (A), 14 (B), and 28 (C). FAD images; arrows point at new hair follicles.

Figure S9. Representative FAD fluorescence intensity images of an area near the wounds of mice from each group, at each imaging day.
**Supplementary Tables**

*Table S1. P-values of one-way ANOVA analysis of the Control group compared to the other groups; confidence level of 95% (P<0.05).*

<table>
<thead>
<tr>
<th>Group</th>
<th>Redox ratio</th>
<th>NADH mean lifetime</th>
<th>Bound NADH fraction</th>
<th>OCTA Branch density</th>
<th>OCTA Mean intensity</th>
<th>SHG width</th>
<th>Wound size</th>
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</thead>
<tbody>
<tr>
<td>Comp. = 0.5%</td>
<td>0.0437</td>
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<td>0.0033</td>
<td>0.0230</td>
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<td>0.0001</td>
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<td>0.4187</td>
<td>0.9491</td>
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</tbody>
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