

# ASSESSMENT OF WOUND HEALING TREATMENTS WITH LONG TERM SKIN CULTURE METHODS AND A NOVEL WOUNDING DEVICE



A. Resek, J. Lenn, A. Amegashie, M. Awawda, A. Vera Collins, J. Volmer, A. Woodson, W. LaBarge

MedPharm, Ltd., 4222 Emperor Blvd, Suite 335, Durham, NC 27703

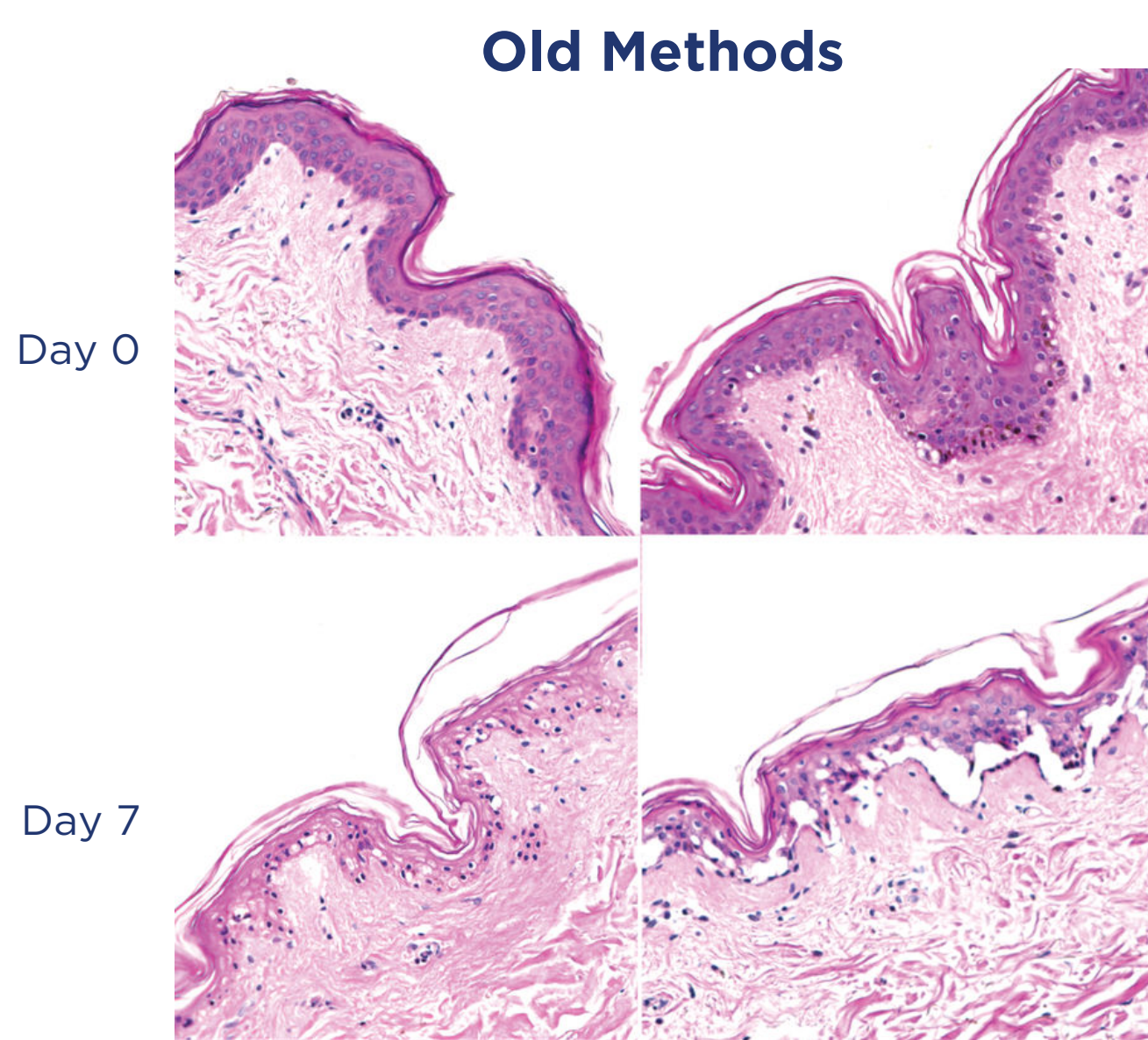
## ABSTRACT SUMMARY

The current methods for studying wound healing rely on a biopsy punch to introduce the wound, and traditional culture methods which are typically not stable past 10 days. The use of a manual punch is labor intensive leading to variable wound depth, high error rate, highly technical training, and significant bench time required to perform experiments. Wound healing studies are further hampered by the time in culture limitations of human skin explants, as wounds cannot go through the phases of wound healing in only 10 days.

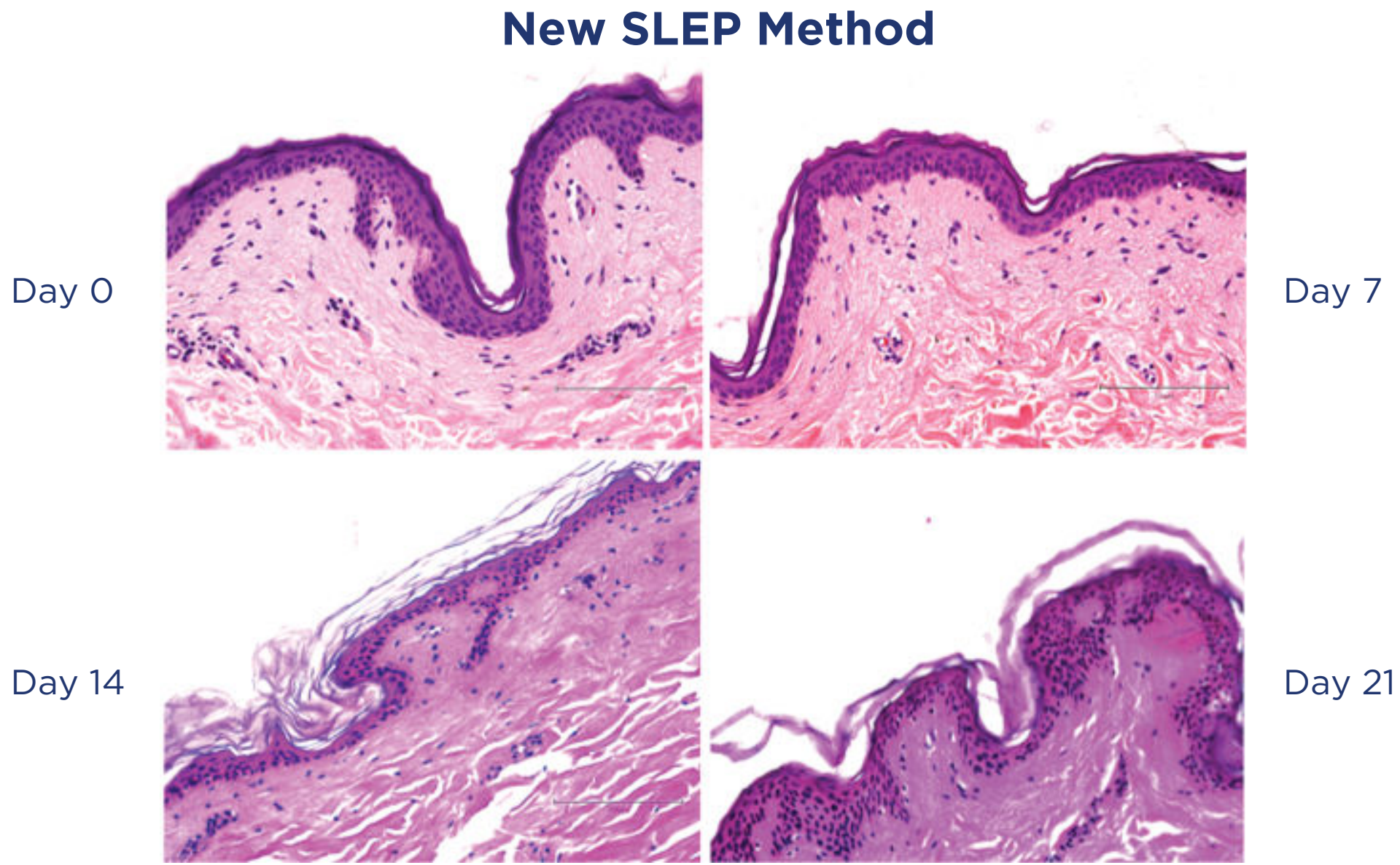
We utilize a combination of three new methods and specialized tools to address these limitations described above. First, we have developed an improved explant culture device. This device allows for more efficient use of the limited amounts of suitable human skin that can be acquired from cosmetic surgery, compared to static vertical diffusion cells (i.e. "Franz cells") or transwells. Second, we have developed a new culture methodology that allows for the culture of explant skin for up to 28 days. Finally, we have developed a skin wounding device to be used in conjunction with the culture device, which reduces reliance on manual wounding, both reducing the amount of time required to wound the skin and reducing the variability between wounds.

This study used both our devices, where 4 skin donors are cultured for 21 days post-wounding in the long-term culture media, using both positive and negative controls. By the end of three weeks, wounds have visibly healed in positive control, negative control, and unwounded skin, with notable differences in the treated group in terms of gross appearance, histology, and gene expression of inflammation and cell signaling (TNF $\alpha$ ), matrix proteins (collagen, involucrum, KRT14), MMPs, and proliferation markers (PCNA, Ki67).

## SKIN LIFE EXTENSION PREPARATION (SLEP)



Methods for long-term culturing of human skin are available in published literature. Using these methods, the skin on day 0 is as expected with healthy cells and a robust epidermis. However, by day 5 cell death is visible in the tissue. At day 7, cell death is prominent and epithelial thinning is now noticeable. Finally, after 14 days of culture there are dead cells, epithelial thinning, and separation of the epidermis from the dermis.

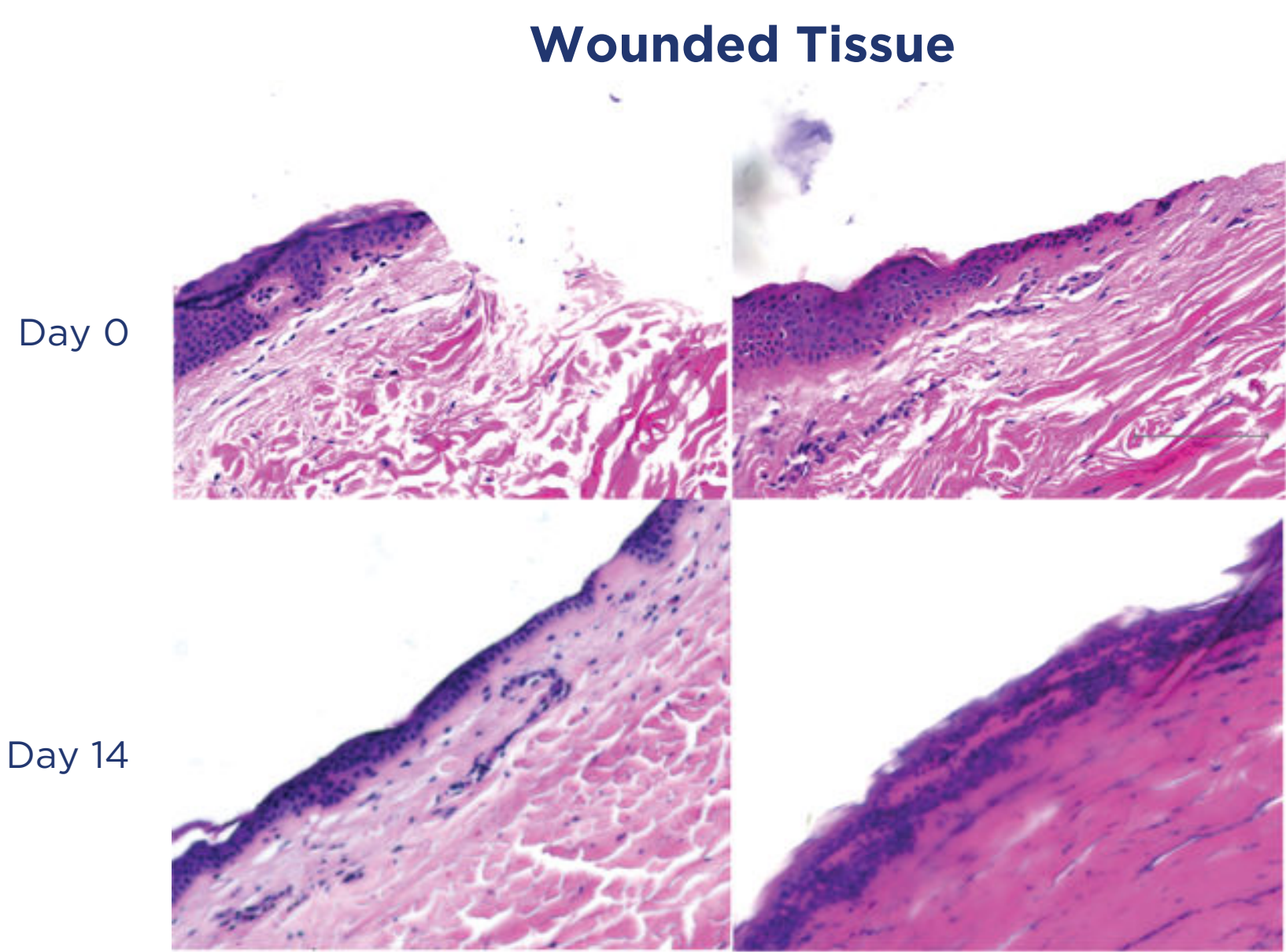


MedPharm's Skin-Life Extension Preparation (SLEP) is the development of a novel culture technique to improve the longevity of cultured skin explants. Using our SLEP protocol, through 21 days of culture a healthy and intact epidermis is visible with less epithelial thinning and minimal cell death.

## NEW WOUNDING METHODS



MedPharm's wounding device was designed to simultaneously introduce 8 wounds with less than 10% variability in wound depth. The wounded skin is mounted to our high-throughput pharmacodynamic device (HTPD) and sustained with our internally developed skin-life extension media. Each wound is isolated to allow for multiple treatment conditions and individual assessment of wound healing.



Tissue wounded on our device harvested immediately after wounding demonstrates a well-defined wound edge. On day 7, the edge of the wound is still visible where the stratum corneum ends. Beyond that shows an infiltration of keratinocytes proliferating across the wound bed. By day 14, the wound bed is nearly completely covered by a thin layer of keratinocytes. Finally, on day 21 the cell layer is much thicker suggesting keratinocyte differentiation has begun. Throughout the 21 days cells within the tissue appear healthy and the rebuilt epidermis displays no separation.

## RESULTS

Unwounded  
Wounded/Untreated  
Wounded + Treatment

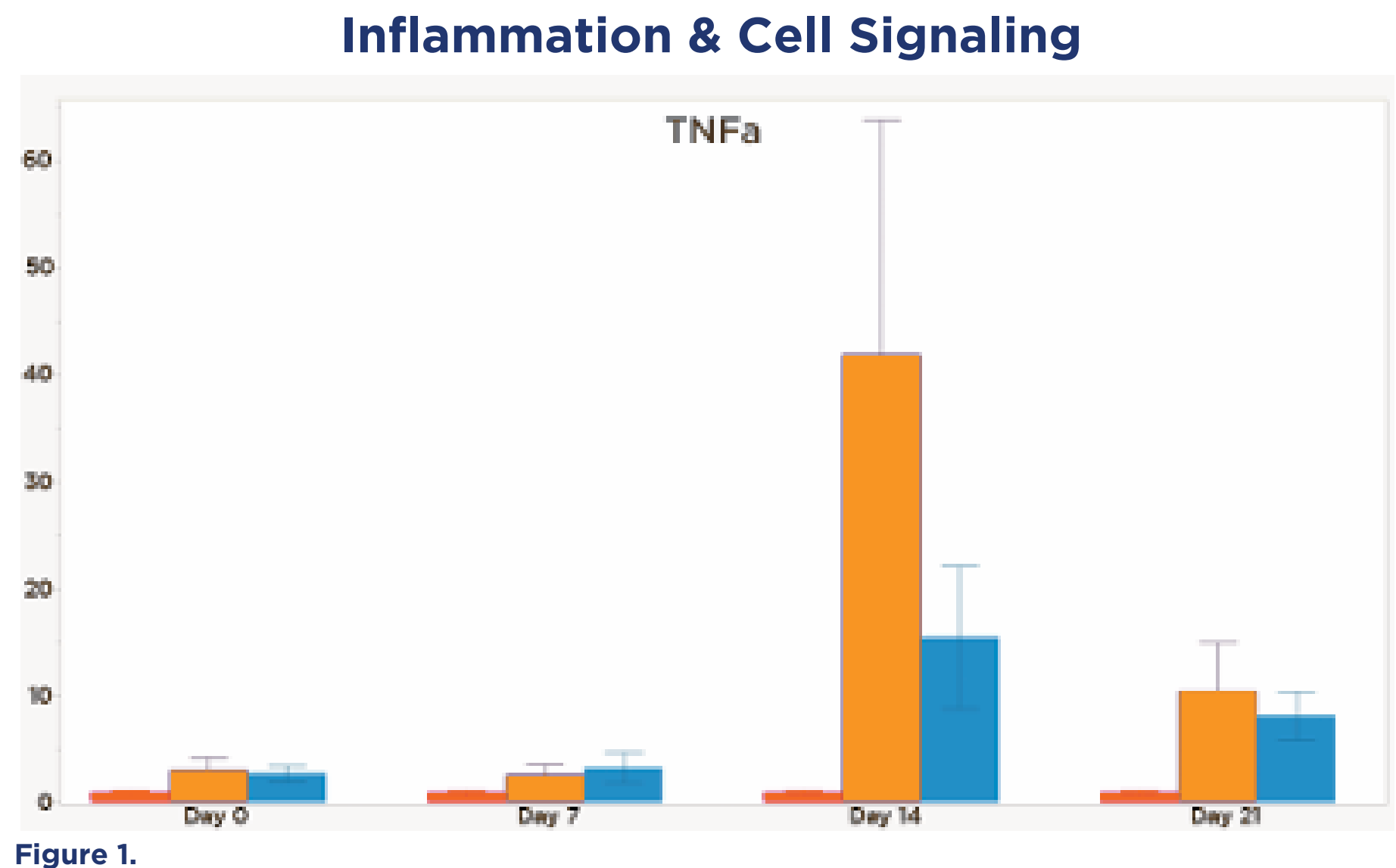


Figure 1.

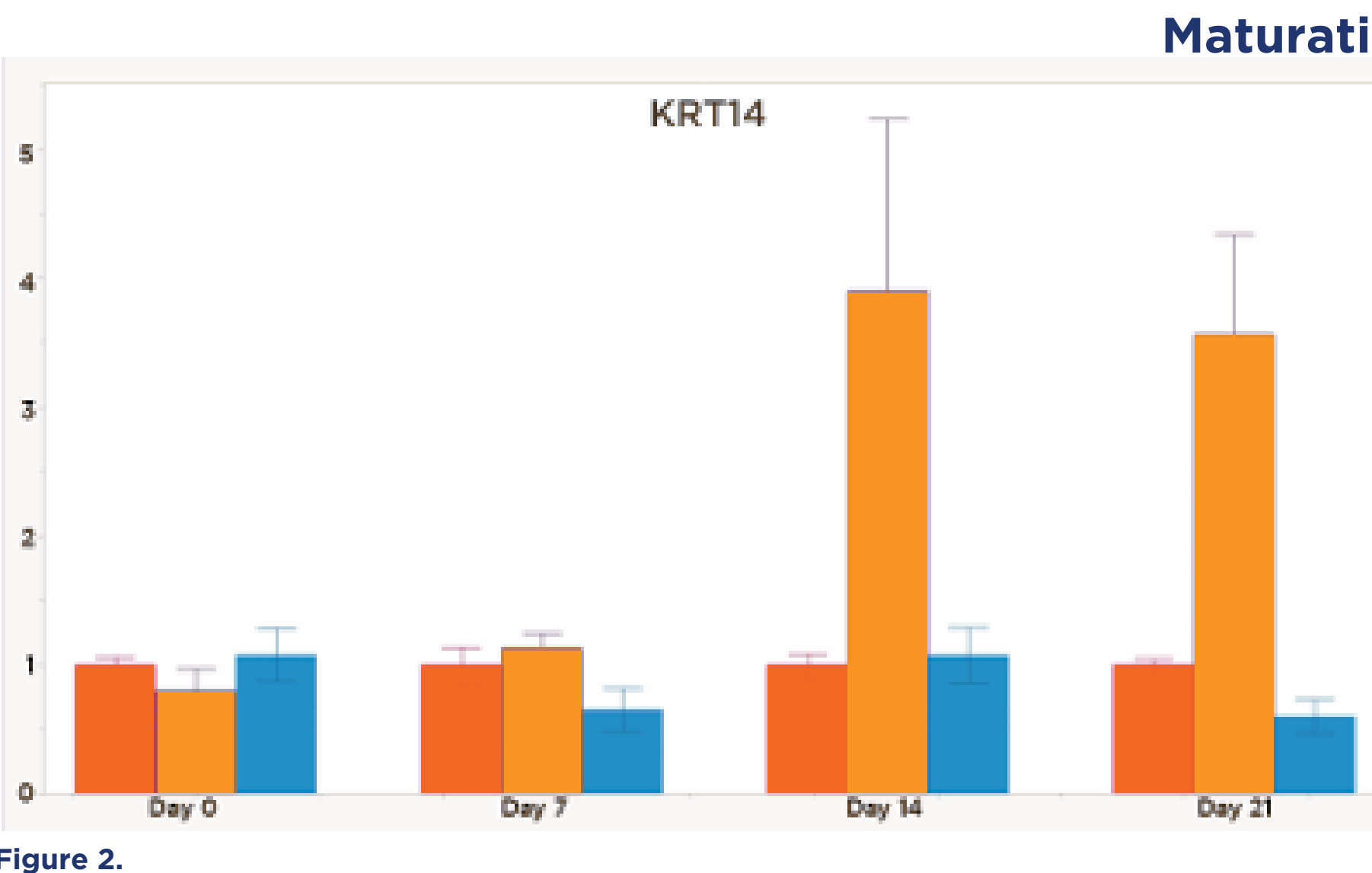


Figure 2.

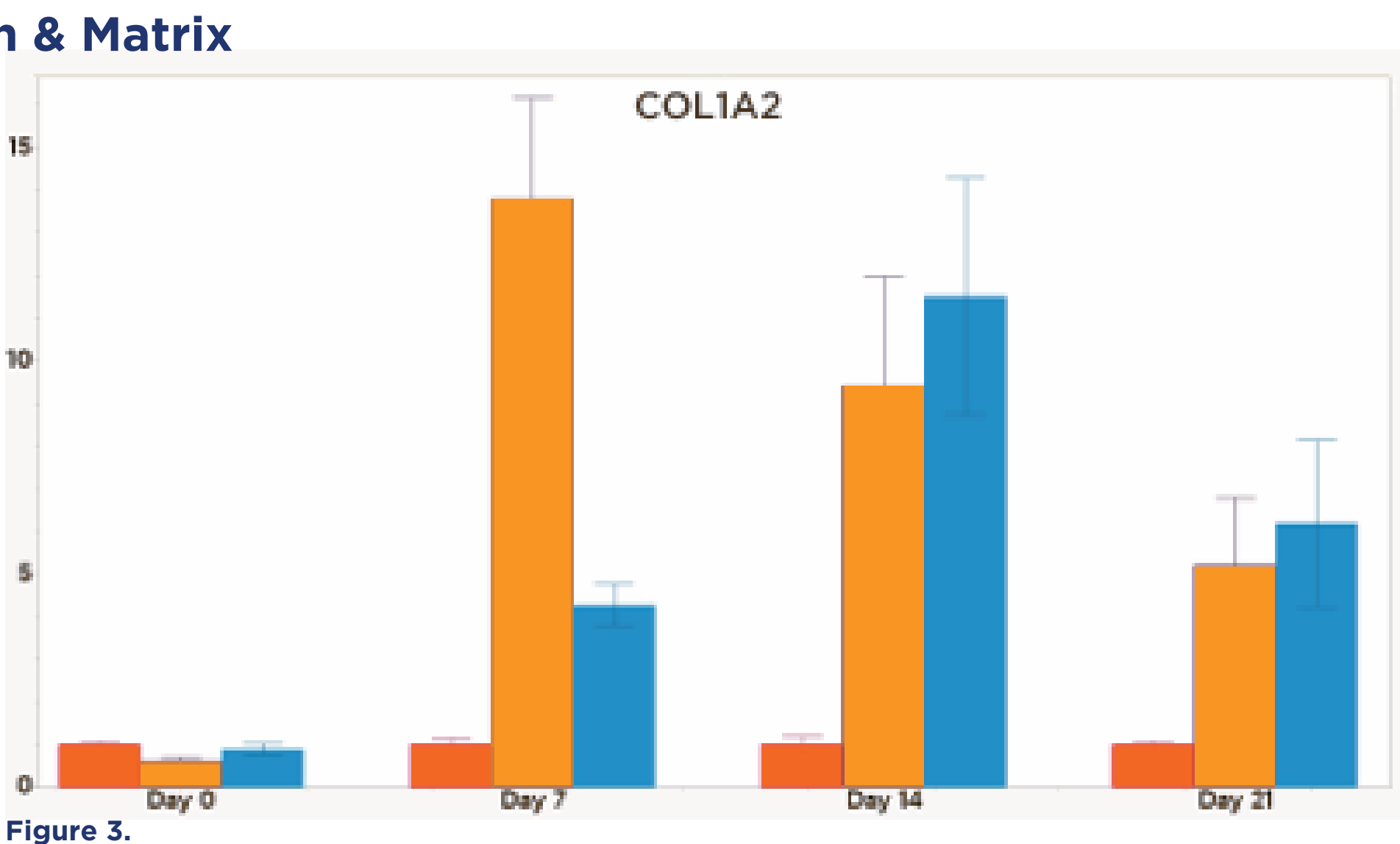


Figure 3.

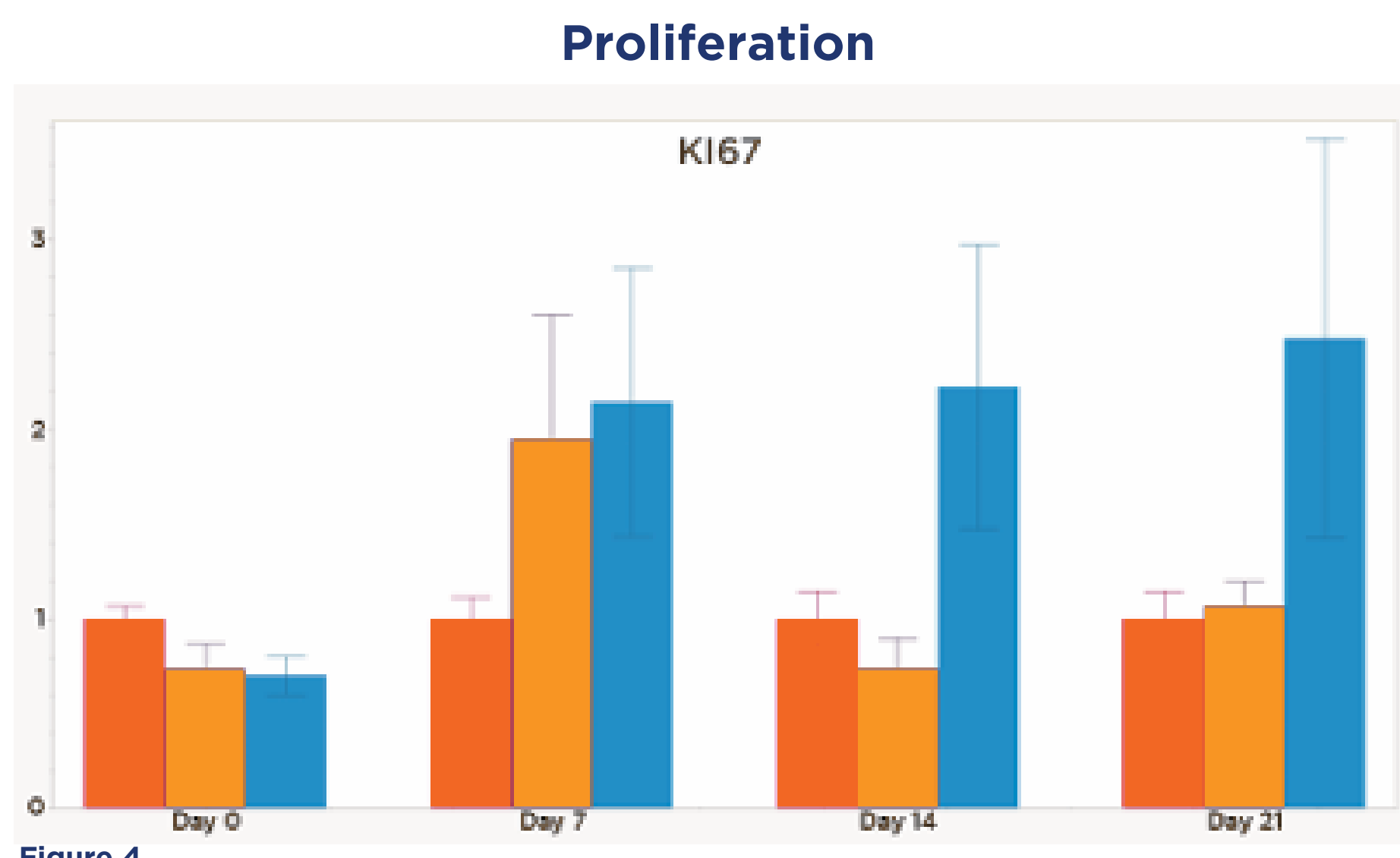


Figure 4.

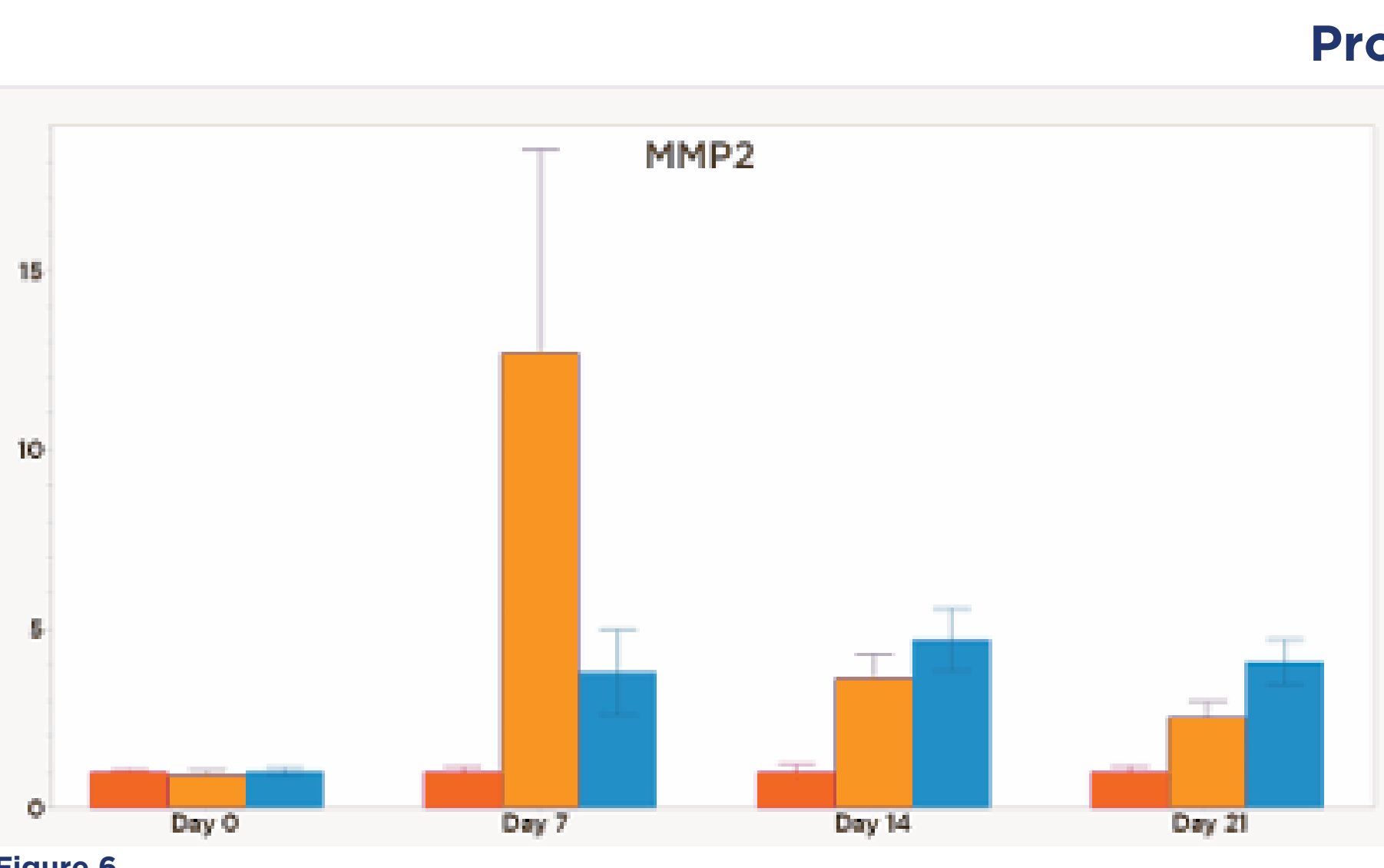


Figure 6.

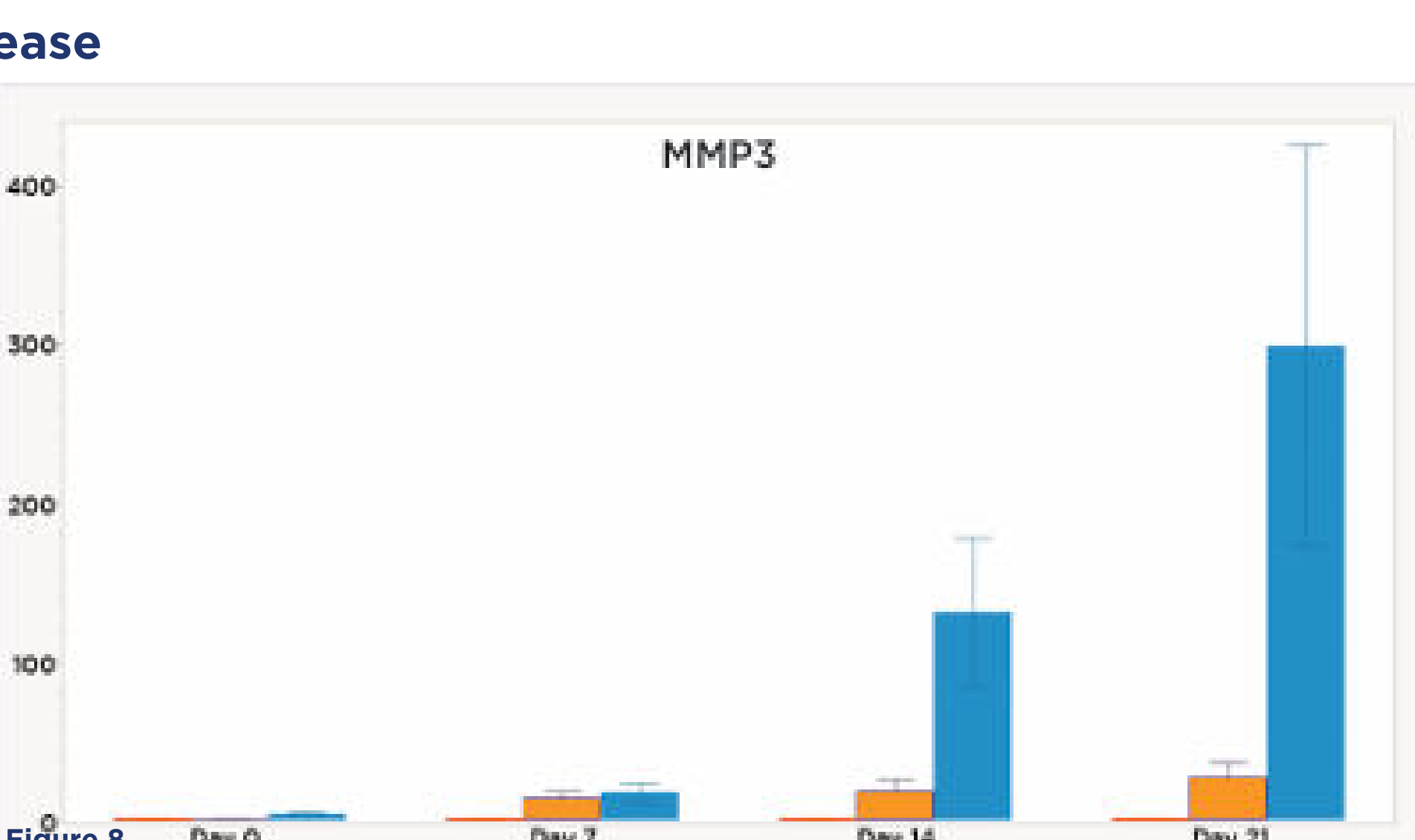


Figure 8.

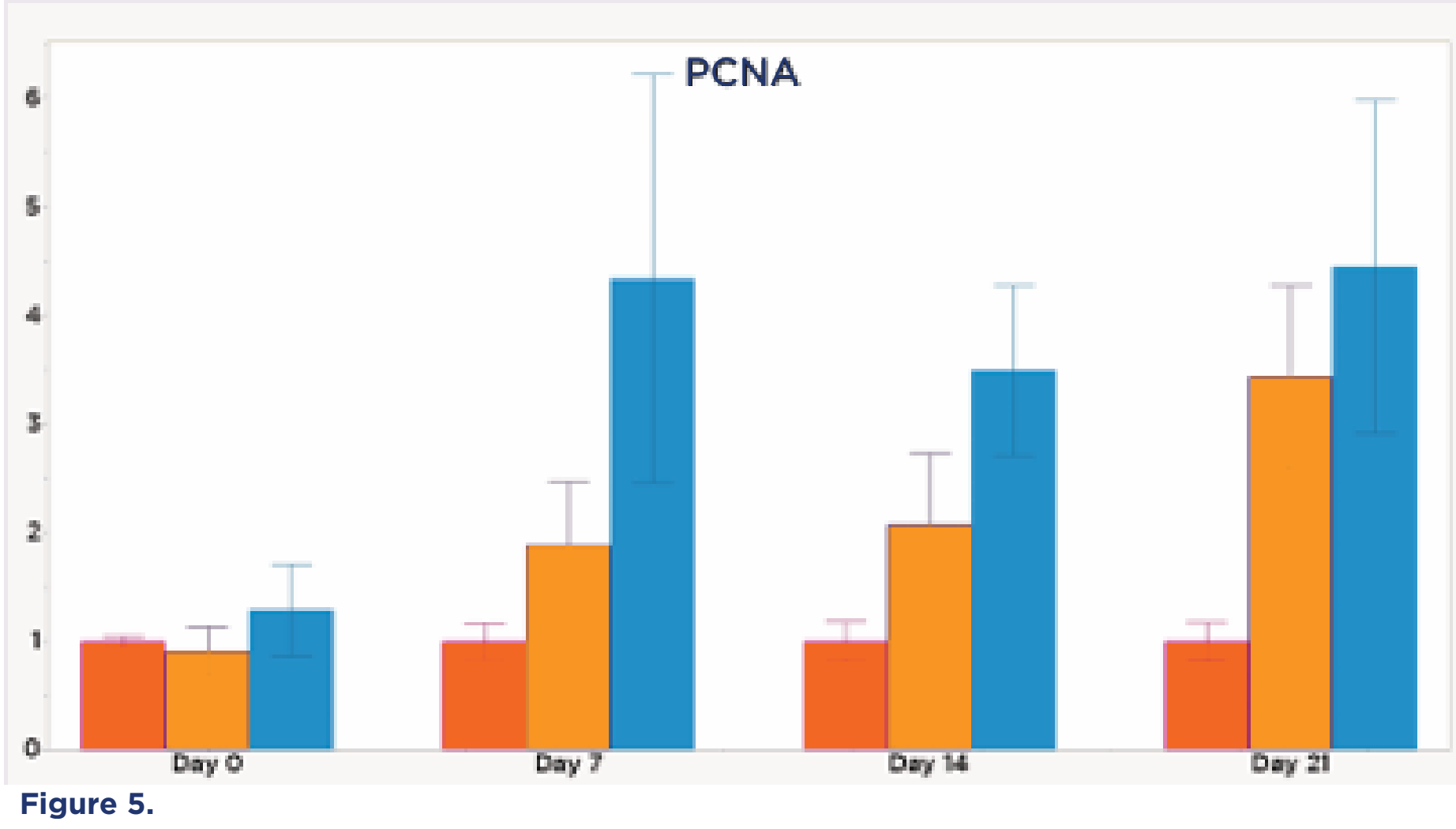


Figure 5.

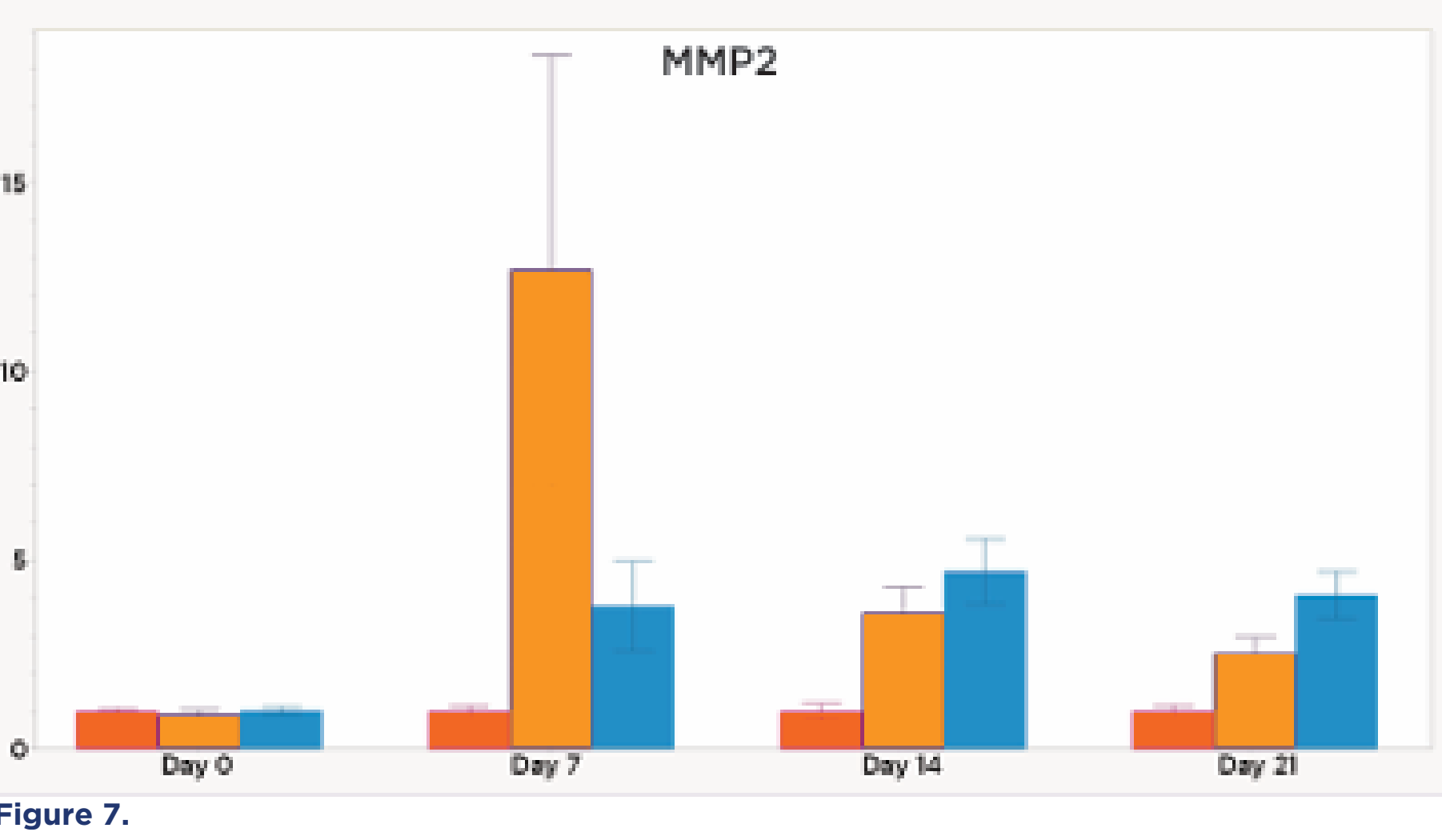


Figure 7.

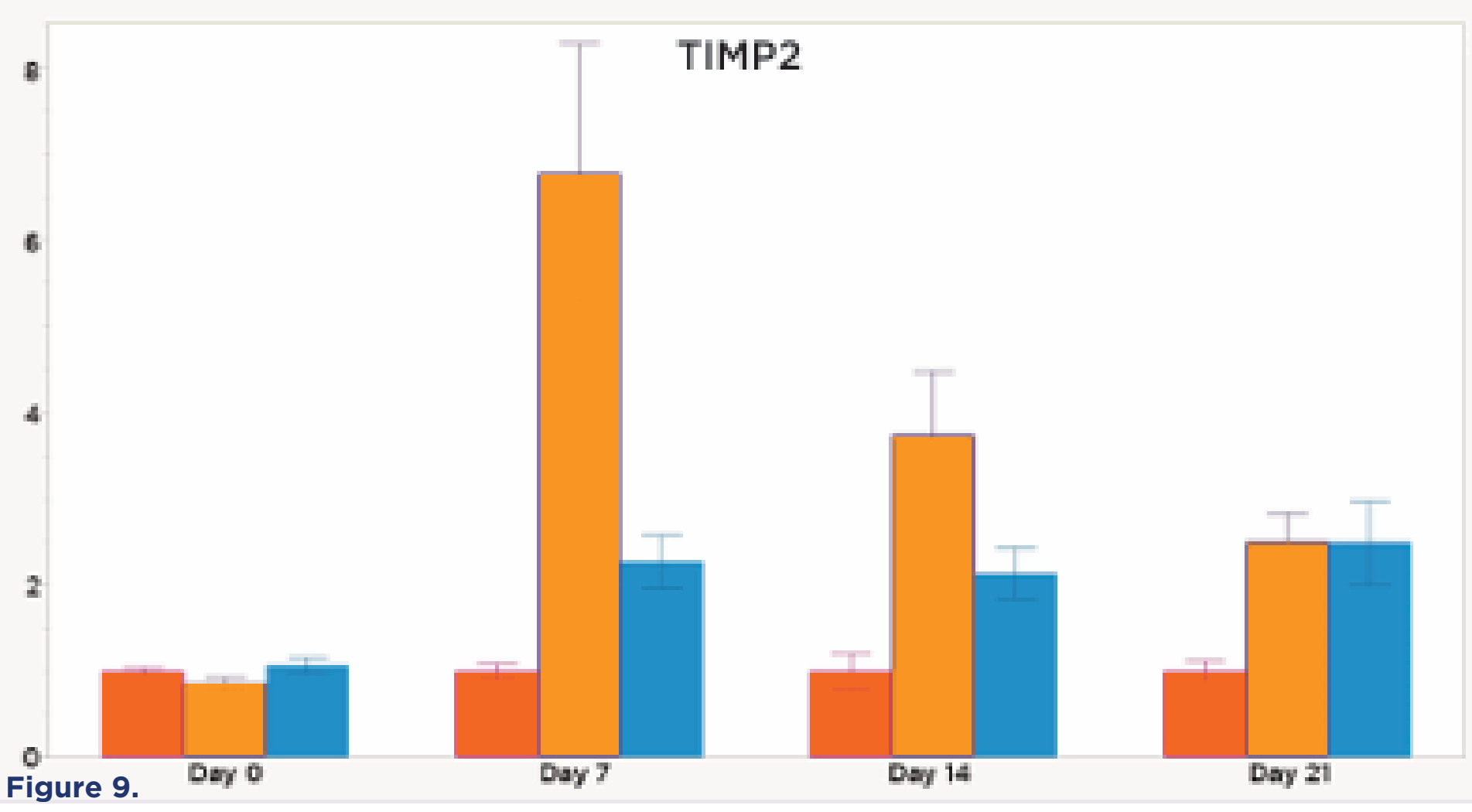


Figure 9.

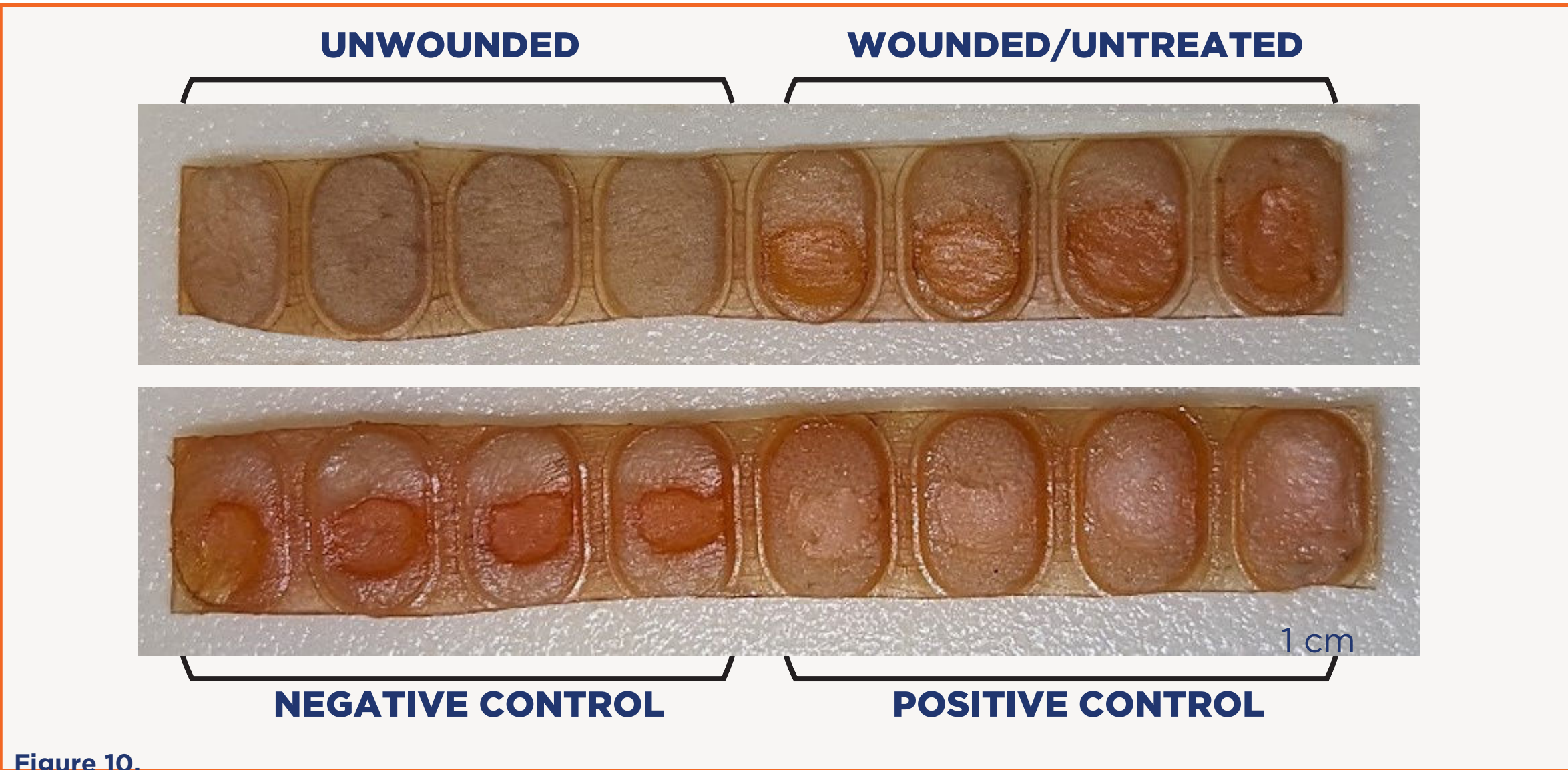


Figure 10.

To assess wound healing treatments with our long-term skin culture methods and novel wounding device, an experiment was performed using human abdominal skin (N=4 donors). After wounding the skin with the wounding device and mounting the samples on the explant culture devices, treatments were applied (n=4 per treatment) and the SLEP method was then employed. At the end of a 21 day culture period, samples were collected and RT-qPCR was completed to compare the treatment groups and investigate specific wound healing hallmarks.

**Figure 1.** TNF $\alpha$  expression was measured to evaluate the inflammation and cell signaling that occurred at each timepoint. Day 14 showed an increase in expression for both treatments with the Wounded/Untreated group displaying a larger increase compared to the Wounded + Treatment group.

**Figures 2 and 3.** KRT14 and COL1A2 expression was assessed to determine the extent of cell maturation and matrix deposition in the wounds. For KRT14, Day 14 showed an increase in expression for the Wounded/Untreated group that was sustained through Day 21 with no change observed in the other treatment groups. For COL1A2, by Day 7, expression for both the Wounded/Untreated and Wounded + Treatment groups was increased with the Wounded/Untreated group being substantially higher. However, expression for the Wounded/Untreated group decreased over the remainder of the experiment and the expression in the Wounded + Treatment group showed a delayed increase at Day 14.

**Figures 4 and 5.** cell proliferation was analyzed through Ki67 and PCNA expression. Ki67 expression increased in both the Wounded/Untreated and Wounded + Treatment groups by Day 7. However, the Wounded/Untreated group expression decreased to a similar expression as the Unwounded group while the Wounded + Treatment group expression was sustained through Day 21. The expression of PCNA also increased at Day 7 for those treatments with the Wounded + Treatment group sustaining expression through the experiment, and the Wounded/Untreated group gradually increasing over time.

**Figures 6-9.** protease expression was measured through the biomarkers MMP2, MMP3, TIMP1, and TIMP2. MMP2 expression increased for both the Wounded/Untreated and Wounded + Treatment groups at Day 7 with the Wounded/Untreated group greatly increasing. However, the expression for the Wounded/Untreated group decreased over the remainder of the experiment while expression for the Wounded + Treatment group remained at the same level. For MMP3, the expression was similar for both treatment groups at Day 7, but the expression for the Wounded + Treatment group substantially increased over the remainder of the experiment while the Wounded/Untreated group remained the same. Both TIMP1 and TIMP2 expressions had similar trends for both treatments with the Wounded/Untreated group showing an initial increase at Day 7, but steadily decreased over the remainder of the experiment. In the Wounded + Treatment group, TIMP1 and TIMP2 increased to a lesser extent at Day 7 but maintained this increased expression throughout.

**Figure 10** shows an example of a donor skin explant after 21 days of culture using the setup and treatments described. The negative control pictured is wounded skin treated with saline.