Graft Incorporation and Microangiogenesis Evaluation via Fluorescence Angiography Following Application of an Acellularized Dermal Matrix

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APWCA
AMERICAN PROFESSIONAL WOUND CARE ASSOCIATION

16th Annual National Clinical Conference

September 7-9, 2017

Loews Philadelphia Hotel | Philadelphia, PA

Disclosures

- None to disclose

Poster

2017

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PURPOSE

To evaluate the incorporation of an acellular dermal matrix graft by evaluating vascularization in a noninvasive manner via fluorescence angiography.

BACKGROUND

Acellular dermal matrix (ADM) grafts have risen in popularity over the previous decade both with increasing brands/types of grafts and also their indications. Very limited research has been performed evaluating the exact level of vascularization within these grafts once applied. Previous studies have assessed ingrowth of vessels via cytoscan, also by sectioning the recently grafted area and staining with hematoxylin and eosin then counting each new lumen.[1] With today's technology of fluorescence angiography, one can evaluate vascular growth not only more accurately but also, and some would argue more importantly, in a more noninvasive manner. To our knowledge, there have not been any studies evaluating graft microangiogenesis via fluorescent angiography.

METHODOLOGY

This study details the case of a 39 year old African American male who contracted necrotizing fasciitis from an infected boil on his right anterior lower leg. After serial debridements of all nonviable tissue and eradication of the infection, an impressive defect remained totaling 195 cm². Due to the patient having no comorbidities and large granular wound bed, he was a prime candidate for this study. A preoperative fluorescence angiography scan was performed and then an ADM graft was applied intraoperatively using the preferred technique as described by the manufacturer. Fluorescence angiography studies were then performed at successive intervals (48hr, 14d, and 21d) to evaluate for vascular incorporation of the graft. The degree of vascular ingrowth was determined based on the date at which the post-application study ingress and maximal point of intensity rates reached that of the pre-application. At day 21, the graft was found to have fully vascularized.

Initial fluorescent angiography peak.

Fluorescence angiography at 48hr post-graft placement.

Fluorescence angiography at 3 weeks post-graft placement with 99% illumination obtained.

Defect pre-graft placement. Note 100% granular base, which is crucial for optimum graft success.

Defect at 48 hours post-graft placement with graft held in place via running suture technique.

Defect at 3 weeks post-grafting.

DISCUSSION

Fluorescence angiography uses a fluorescein dye, most commonly indocyanine green (ICG), to allow visualization of tissue perfusion. ICG is water soluble and filtered through the liver, making it ideal for various patients with renal issues. It has been used for decades for a variety of medical uses, namely to measure cardiac output and ocular assessments. ICG absorbs light at near-infrared levels and produces a fluorescent image in real-time.

Due to vast technological advancements, physicians have multiple options when it comes to choosing acellular matrix products to assist in wound healing. These grafts function generally as a scaffold to assist in cellular proliferation and migration, as well as a delivery system for various factors that direct capillary growth, as well as fibroblast and endothelial invasion.[2] They can be comprised of porcine, bovine, After the graft has been placed it begins to incorporate and interact with the host bed by forming a vascular network between the two. This process is known as inosculation. The graft begins to turn pink as the blood flow is restored to the graft. While the exact mechanism of graft revascularization was not evaluated during this study, there are three well represented theories in the literature to explain how the process is accomplished, the effects of which can all be evaluated via fluorescence angiography.

Connection of graft and host vessels

According to this theory, vascularization is restored via the original vessels within the graft. Via inosculation, vascularization is accomplished by an anastomoses formed between the recipient bed and the graft. There have been previous studies evaluating this method via India ink and a porcine graft. Ink was injected into vessels after graft placement and continuously monitored. After several days, there was evidence of ink spreading between the graft and the wound bed suggesting interaction between the vessels of the graft and the wound bed.[3,11]

Formation of new vascular channels

The new vascular channel theory holds that the graft is perfused by the formation of new vessels as it interacts with the graft bed. Converse and Rapport evaluated grafting in humans and discovered an early connection of graft and host vessels.[3] A later study supported this theory using rat models and showed that the final vasculature of the graft stemmed from ingrowing vessels from the host. To support the new vascular channel theory, Converse used diphosphopyridine nucleotide diaphorase to highlight the difference between the new active vessels and the inactive, previously existing graft vessels.[4] Using this enzyme found in active, healthy red blood cells they were able to identify degenerative changes in the older vessels within the graft vasculature. Although this theory supports the growth of new vessels, Zarem posited that the old vessels were not discarded but served as conduits for neovascularization of the graft from the host bed.[5]

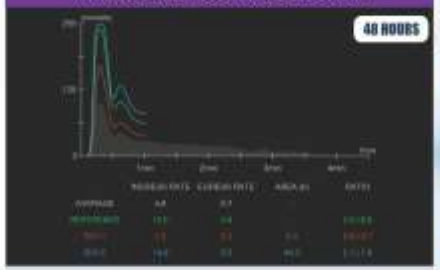
Combination of old and new vessels

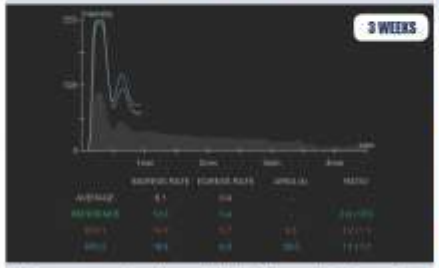
This theory ties together the two mentioned above. It postulates that existing vessels with in the graft are recycled, as well as new vessels are created. The two pathways restore circulation simultaneously to the graft as it interacts with the graft bed.[3,5]

CONCLUSION

The present study placed the several advantages of fluorescence angiography on display. Not only were the vascular components of graft incorporation able to be precisely determined, but also, it was done so in a noninvasive manner. Additionally, complete vascular incorporation was noted at three weeks, which is earlier than the current literature has suggested.

MAX INTENSITY COMPARISON GRAPH

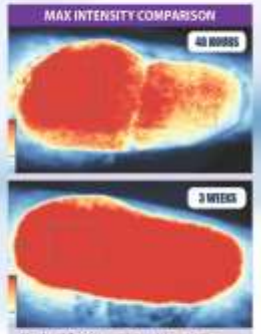




Fluorescence angiography maximal intensity graphs. The green line represents the periwound which is 100% vascularized, this is the goal for the grafted areas to obtain. The blue line represents the Region of Interest (ROI) #1 which is over the large proximal aspect of the graft which was noted to be the most vascular preoperatively.

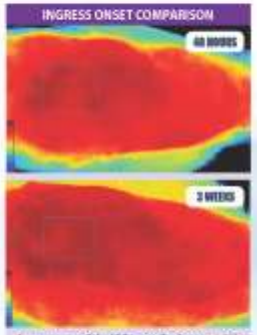
The orange line represents the ROI #2 which was determined to be the least vascular area preoperatively. Note at week 3, the least vascular area exceeds that of the reference, indicating complete vascular incorporation of the graft.

MAX INTENSITY COMPARISON



Maximal fluorescent intensities at 48hr and 3 weeks.

INGRESS ONSET COMPARISON



Ingress rate of the ICG to grafted area at 48hr and 3 weeks. This is a direct measurement of blood flow.

REFERENCES

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Review

- Graft incorporation
  - Revascularization
    - Residual host vessels
    - New vessels
    - Combination
  - Recellularization
    - Graft scaffold
    - Migratory inflammatory cells

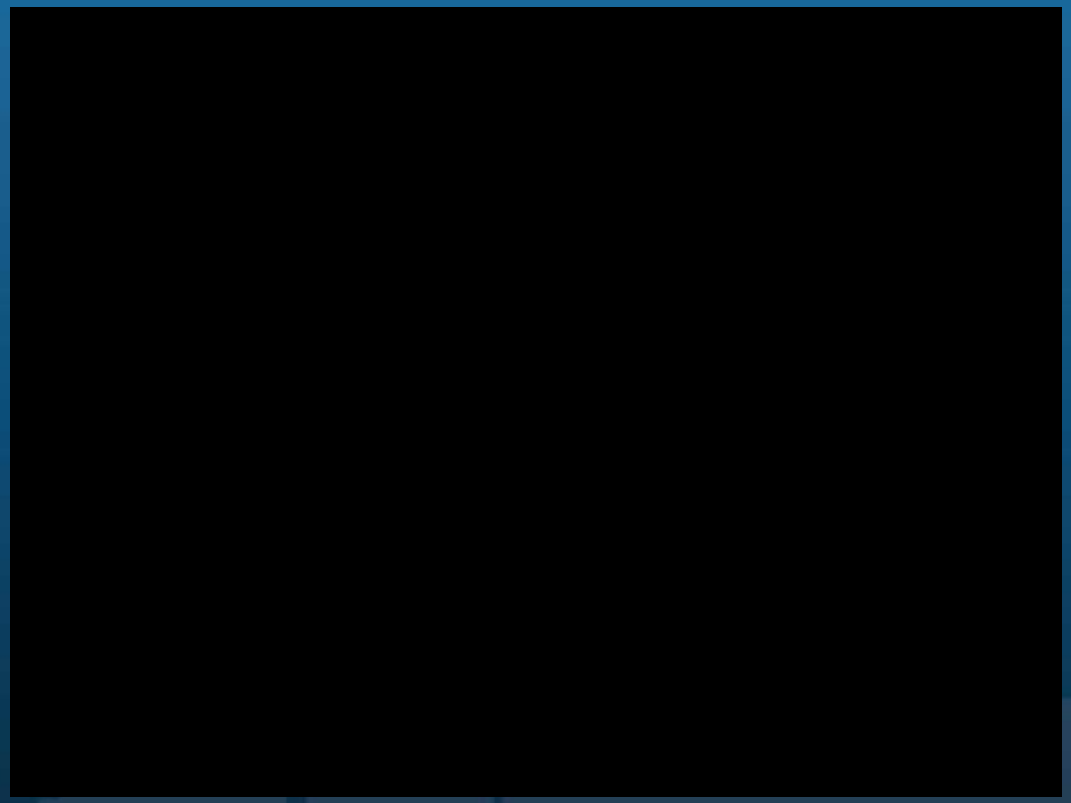
- Fluorescent angiography
  - Indocyanine green (IcG)
    - Hepatic metabolism
  - Uses:
    - Colorectal surgery, plastics, otolaryngology, podiatry, ophthamology, etc.

Case Presentation

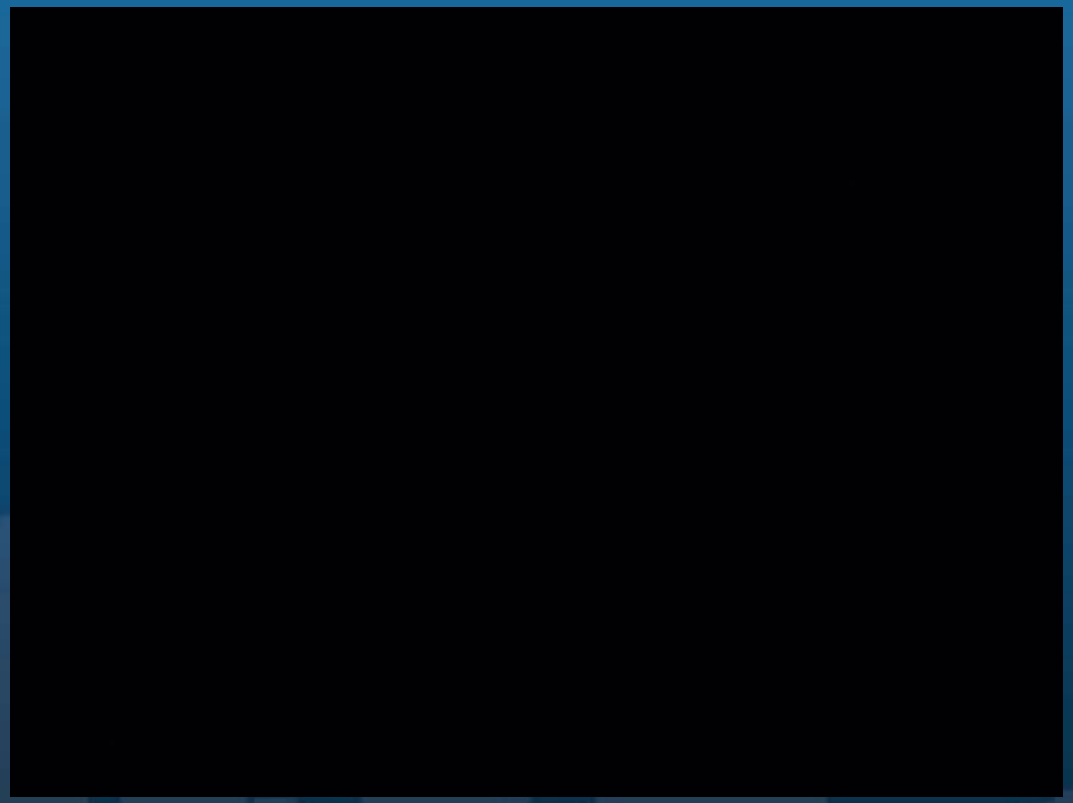
- 39 African-American male
- Necrotizing fasciitis- Right lower leg
- Total defect size 192cm^2
- Negative PMH

Flourescent Angiography

- 48hrs

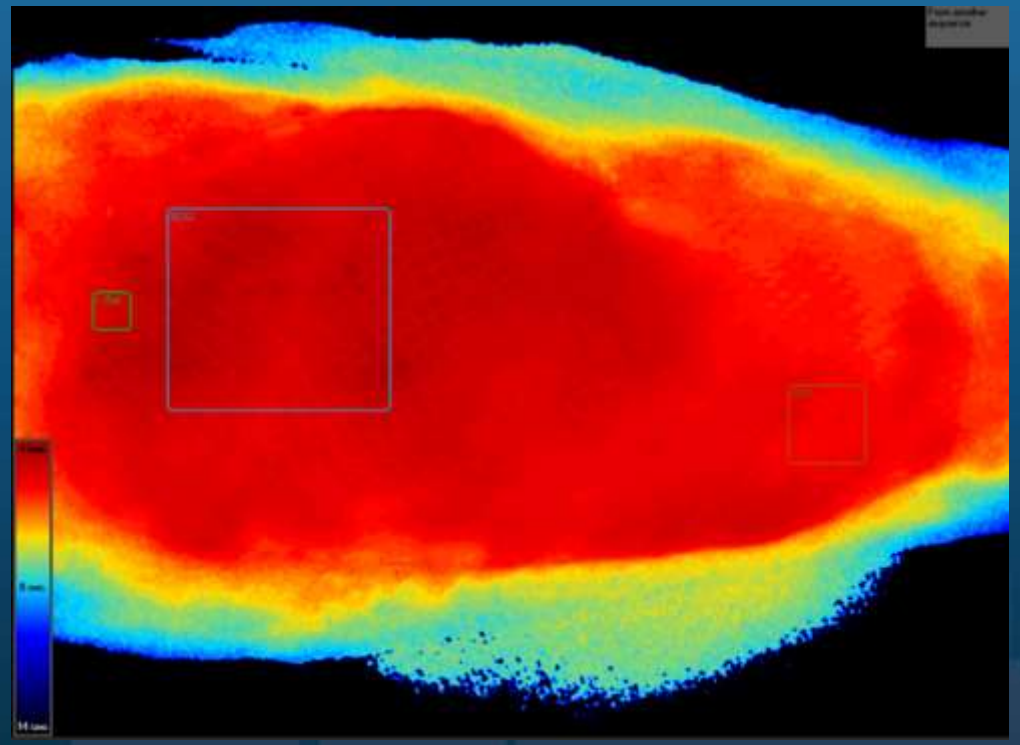


- 3 weeks

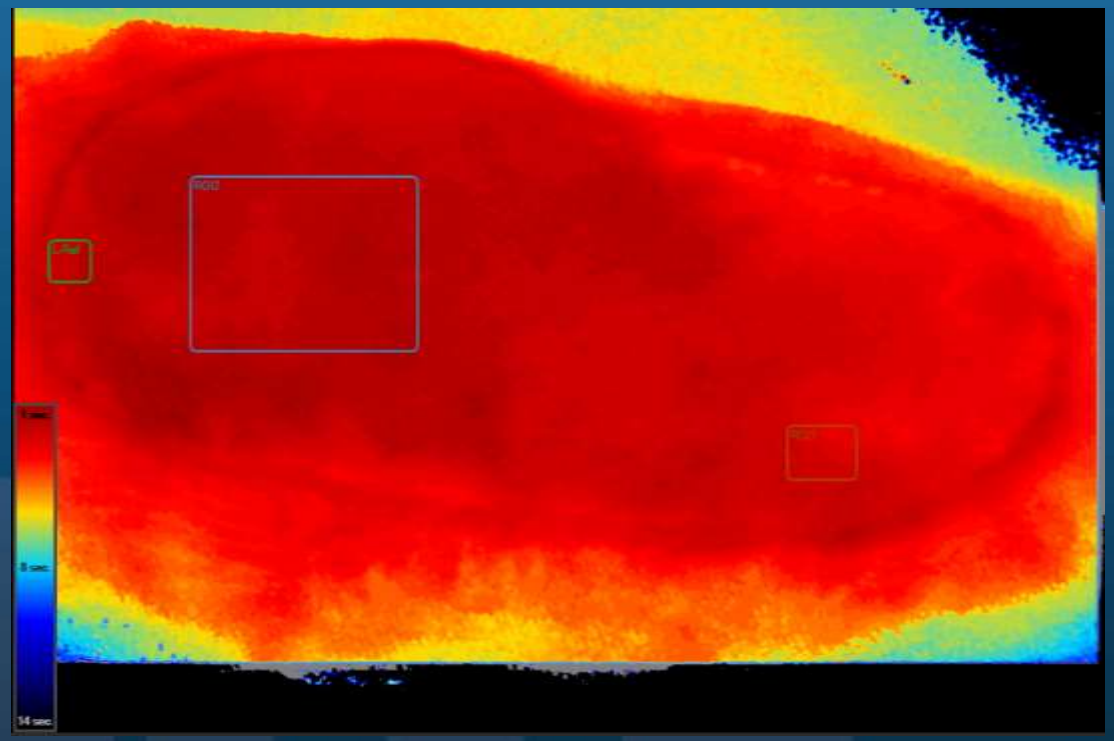


Rates of Ingress

- 48 hours

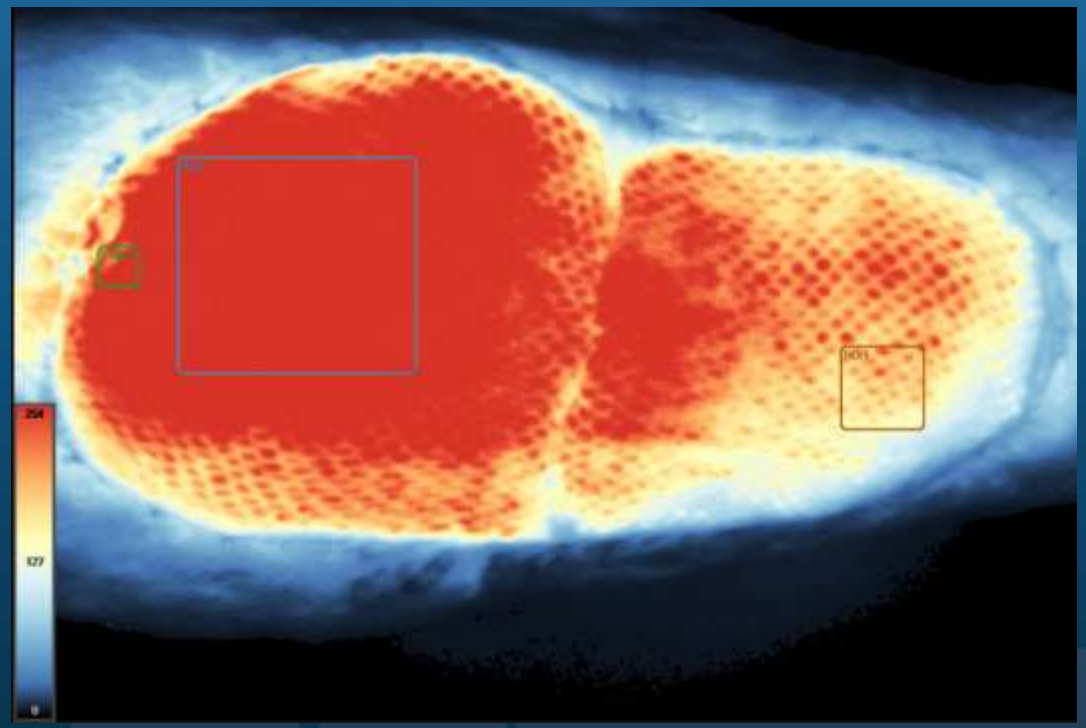


- 3 weeks

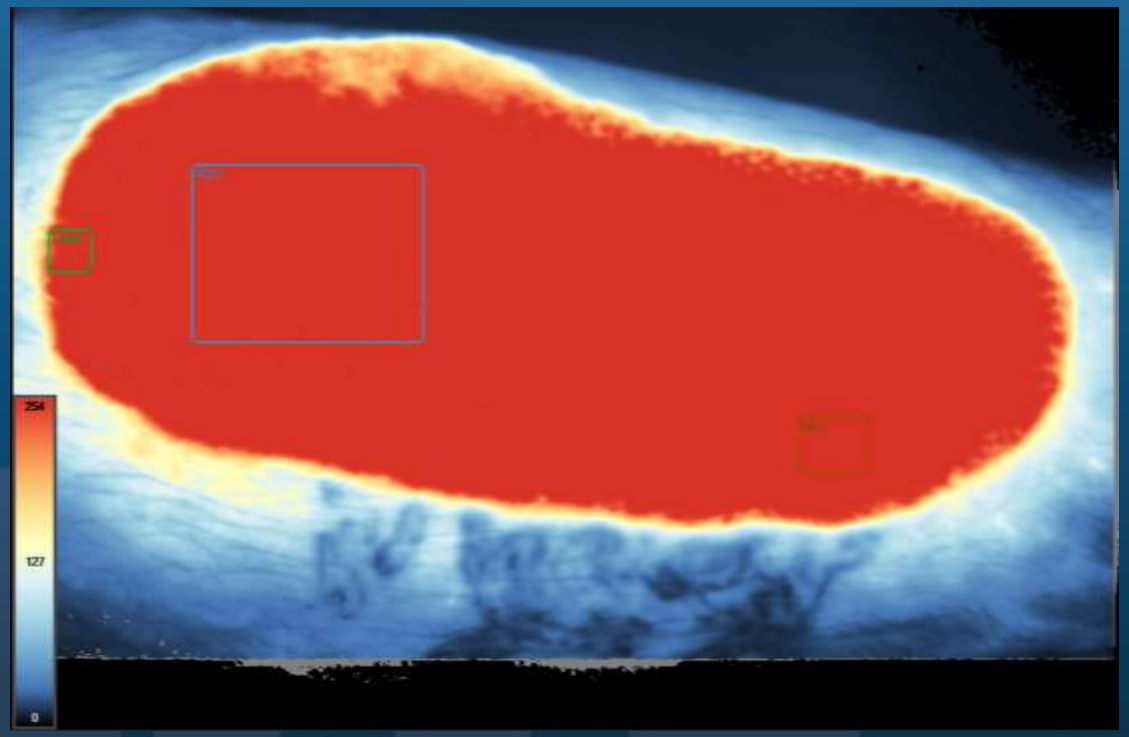


Maximal Points of Intensity (MPI)

- 48 hours

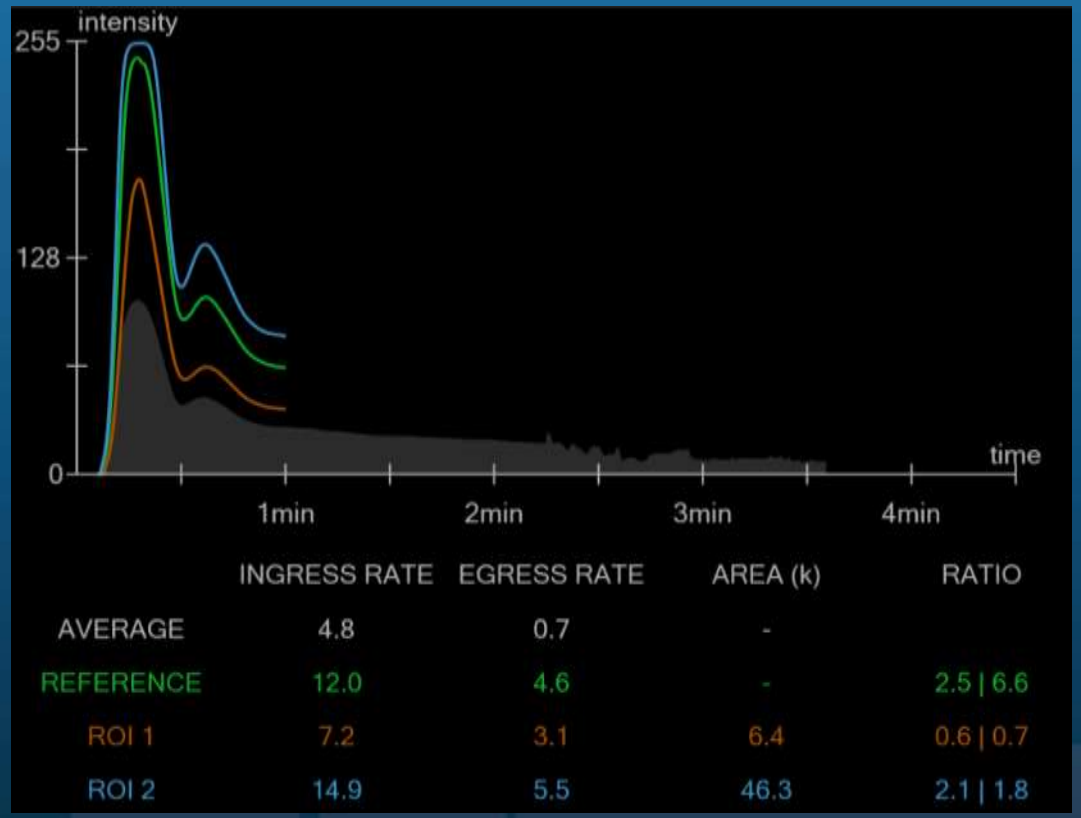


- 3 weeks



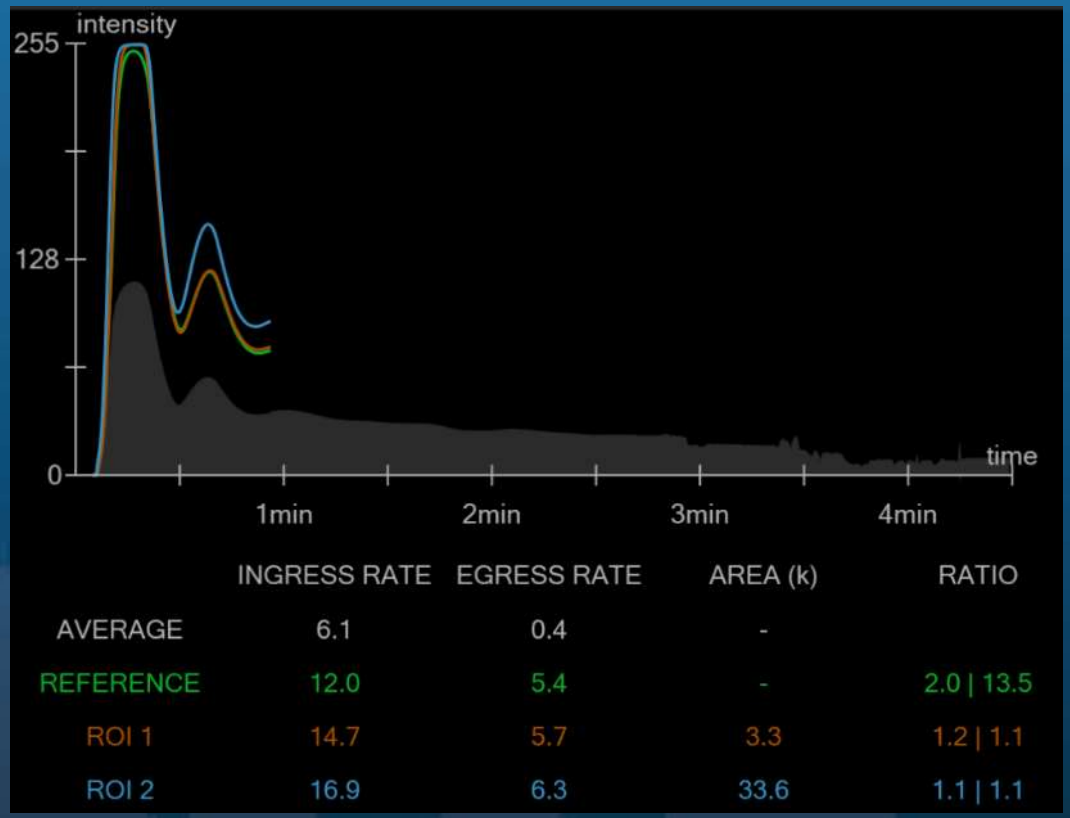
Conclusion

- 48hr



	INGRESS RATE	EGRESS RATE	AREA (k)	RATIO
AVERAGE	4.8	0.7	-	
REFERENCE	12.0	4.6	-	2.5 | 6.6
ROI 1	7.2	3.1	6.4	0.6 | 0.7
ROI 2	14.9	5.5	46.3	2.1 | 1.8

- 3 weeks



	INGRESS RATE	EGRESS RATE	AREA (k)	RATIO
AVERAGE	6.1	0.4	-	
REFERENCE	12.0	5.4	-	2.0 | 13.5
ROI 1	14.7	5.7	3.3	1.2 | 1.1
ROI 2	16.9	6.3	33.6	1.1 | 1.1

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