Vivaderm™
REGENERATIVE TISSUE MATRIX

Enhanced Regeneration with Less Risk

Summary
Vivaderm™ Regenerative Tissue Matrix (RTM) is a collagen matrix allograft derived from human skin that has been aseptically processed and terminally sterilized to preserve the native collagen microstructure while removing the epidermis and dermal cells. A sterile collagen matrix devoid of cells and cellular debris delivers better support of cellular remodeling in the graft and reduces potential adverse immune responses. Vivaderm has a bilayer structure comprised of a dermal layer that allows for recellularization and subsequent revascularization, and a basement membrane that prevents adhesion formation.

Three animal studies were conducted to investigate tissue regeneration (integration, recellularization, and revascularization) and the inflammatory responses of Vivaderm at short and longer-term time points. Histology of the ACD implants was assessed by independent veterinary pathologists. These *in vivo* studies demonstrated the ability of Vivaderm, a safer sterile graft, to integrate with host tissue and promote new tissue formation.

Study 1: Four-day Implantation of Vivaderm™ in Rat Subcutaneous Model
The immediate host response to Vivaderm was investigated in a study in which Vivaderm was implanted subcutaneously in Sprague Dawley rats for four days. A dorsal midline incision, approximately 2 cm long, was made under anesthesia and subcutaneous pockets were created by blunt dissection. Vivaderm grafts measuring 1 cm x 1 cm from three different donors were sutured into the subcutaneous pockets. For comparison, non-irradiated ACD grafts were also implanted to assess the impact of gamma irradiation on the host response.

The grafts were explanted at Day 4, an early time point chosen to avoid the confounding effects of xenograft (i.e. from different species) rejection, and sent to an independent laboratory for histological processing and evaluation. A veterinary pathologist analyzed the tissue samples microscopically for local reaction to the implanted grafts according to ISO 10993-6 guidelines.

Gross examination of the surgical sites for both non-irradiated and Vivaderm revealed the implants were stably embedded within the subcutaenous tissue (Figure 1). There were no signs of infection or inflammation.

Histologically, a mild inflammatory response was noted as expected for a xenogeneic transplant, with no difference between the non-irradiated graft and Vivaderm. As shown in Figure 2, cellular penetration is evident by blue nuclear staining in both grafts, indicating rapid cellular infiltration as early as four days post-implantation. The conclusion from the study is that Vivaderm supports rapid cellular infiltration, and gamma irradiation of the matrix has no negative impact on cellular infiltration.

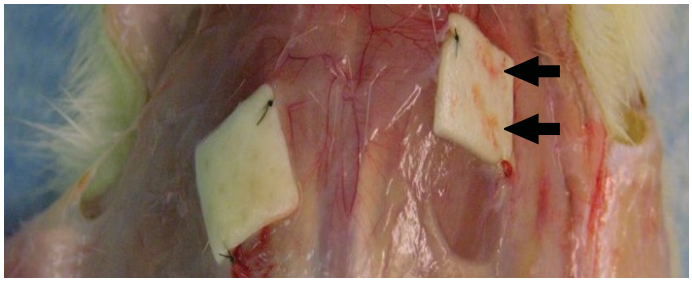

Figure 1. Vivaderm grafts at time of explantation. Left: Non-irradiated graft implanted dermal side down. Right: Irradiated graft implanted basement membrane down. Faster revascularization occurs on the dermal side as evident on the implant (arrows) and the basement membrane limits adhesion formation .

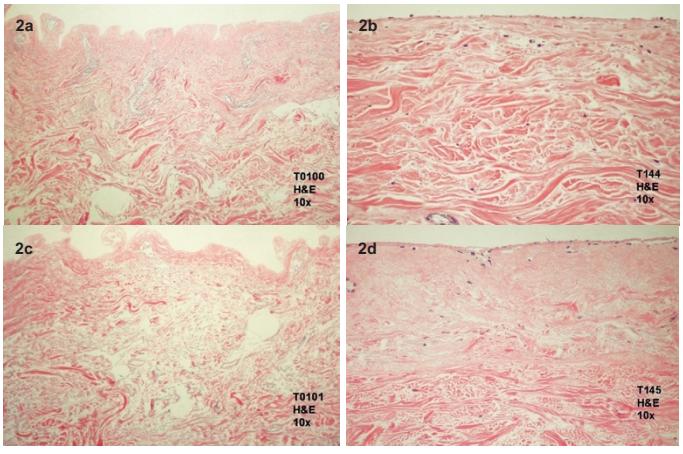

Figure 2. Early *in vivo* response to ACD implantation. H&E histology of ACD pre-implantation and after four days of implantation in rat subcutaneous model. Figure 2a: Non-irradiated graft pre-implantation. Figure 2b: Non-irradiated graft at four days. Figure 2c: Irradiated graft pre-implantation. Figure 2d. Irradiated graft at four days. No differences observed between non-irradiated and irradiated grafts. Post-implantation cellular infiltration is observed in figures b and d (blue punctate staining).

Russell Health™
Regenerative Medicine is our Specialty

Vivaderm™
REGENERATIVE TISSUE MATRIX

Study 2: Three-month Implantation of Vivaderm™ in Rat Subcutaneous Model

The longer-term response to Vivaderm was investigated in a similar subcutaneous implant model in Sprague Dawley rats. To avoid xenogeneic rejection of Vivaderm in a long-term rat study, rat ACD was produced using the identical processing steps as human Vivaderm. For comparison, non-irradiated ACD was also implanted to demonstrate the impact of gamma irradiation on the host response.

Implants were analyzed at 1, 4, and 12 weeks post-implantation (n=5 rats/group). The explanted grafts were sent to an independent laboratory for histological processing and analysis. A veterinary pathologist evaluated the tissue samples microscopically for tissue reaction to the implanted grafts.

Gross examination of the surgical sites for both grafts (non-irradiated and irradiated rat ACD) revealed no signs of infection or inflammation. Histologically, no inflammatory response was noted in either the non-irradiated rat ACD or irradiated rat ACD. An increasing front of cellular infiltration was observed to progress into the midsubstance of the graft over time (Figure 3). As shown in Figure 4, neovascularization and cellular penetration of spindle-shaped fibroblast cells were evident within both graft types indicating host-cell driven tissue remodeling with no impact of gamma irradiation.

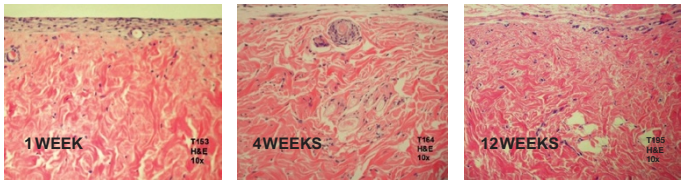

Figure 3. *In vivo* response to ACD implantation in the rat subcutaneous model. H&E histology of ACD after 1 to 12 weeks of implantation. Over time, increasing cellular infiltration and revascularization through the graft is evident.

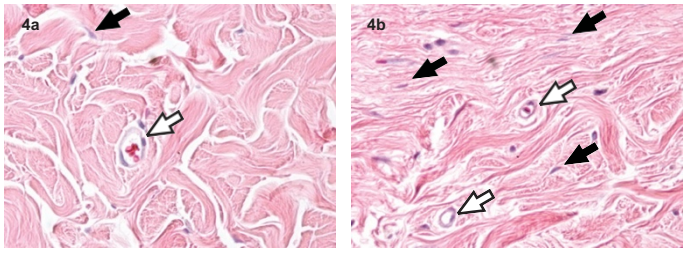

Figure 4. H&E histology at 12 weeks post-subcutaneous implantation of rat ACD into rat (20X). Histology shows clear neovascularization (white arrow) and penetration of spindle-like fibroblast cells (black arrow) within the scaffold. Figure 4a: Non-irradiated rat ACD. Figure 4b: Gamma-irradiated rat ACD.

Study 3: One-month Vivaderm™ Rabbit Integration Model

To study the ability of Vivaderm to integrate, revascularize, and remodel following transplantation, a rabbit model was used. This was a xenogeneic model of human ACD sutured into the abdominal wall of New Zealand white rabbit hosts. Both Vivaderm and non-irradiated ACD were tested (n=8/group). For comparison, an aseptic acellular dermal matrix, Alloderm®, was also included (n=2). At surgery, an incision 7 - 8 cm in length was made in the abdomen, and the skin was resected by blunt dissection. One 3 x 7 cm full thickness abdominal wall defect was made through the muscle and peritoneum as shown in Figure 5a. ACD implants measuring 3 x 7 cm were sutured to the muscle using non-absorbable suture, with the basement membrane side facing the internal organs as shown in Figure 5b. The skin was then closed with absorbable sutures. The rabbits were observed daily for general clinical health and sacrificed one-month post-implantation. The implanted grafts were surgically exposed and gross observations recorded. Explants were sent to an independent laboratory for histology and analysis. A veterinary pathologist evaluated the tissue samples for local tissue response to the implanted grafts and scored inflammatory cellular and regenerative responses using a 4-point grading scheme (0= absent to 4= marked or severe).

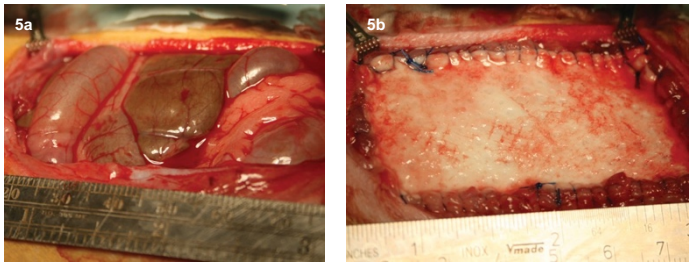

Figure 5. Surgical implantation of ACD into rabbit model. Figure 5a: 3 x 7 cm full thickness defect created in abdominal wall. Figure 5b: Implants were sutured into the defect with the basement membrane side of the ACD facing the internal organs as a barrier to adhesion formation. Note the dermal side, visible in 5b, absorbs blood readily.

Gross examination of the surgical sites for all grafts revealed no signs of infection or inflammation and no defects in the abdominal wall. The implants demonstrated good integration into the abdominal wall and subcutaneous tissue and showed evidence of vascularization with blood vessels on the peritoneal side of the implanted material (Figure 6). None of the animals developed adhesions to the ACD. A small adhesion to the suture material was observed occasionally in all groups.

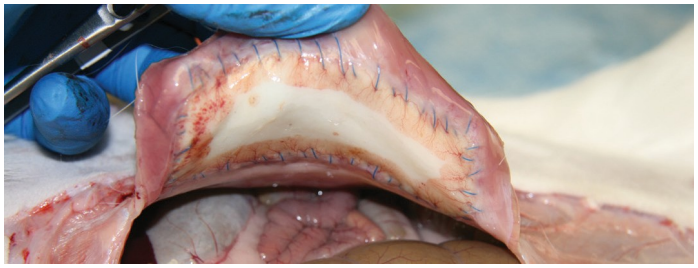

Figure 6. Vivaderm implant after 1 month *in vivo*. Implants were fully integrated into the host tissue and showed evidence of vascularization with blood vessels on the peritoneal side of the implanted graft.

Histologically, review of H&E staining showed a mild inflammatory cellular infiltration that was lymphoctic in nature in Vivaderm and Alloderm. This was expected as the human allografts are xenogeneic to the rabbit model. The graftsshowed new tissue formation, neovascularization, and cellular penetration of spindle-shaped fibroblast cells aligning within longitudinal bundles of collagen, similar to what occurs in normal fascia. Cellular infiltration was present

Russell Health™
Regenerative Medicine is our Specialty

throughout the tissue. A clear remodeling front was evident leaving newly formed collagenous tissue in its wake (Figure 7). Even at this early time point, there was cellular infiltration and remodeling deep into the midsubstance of the graft. Considerable neovasucularization was present throughout the zone of remodeling, which is associated with the new collagen deposition.

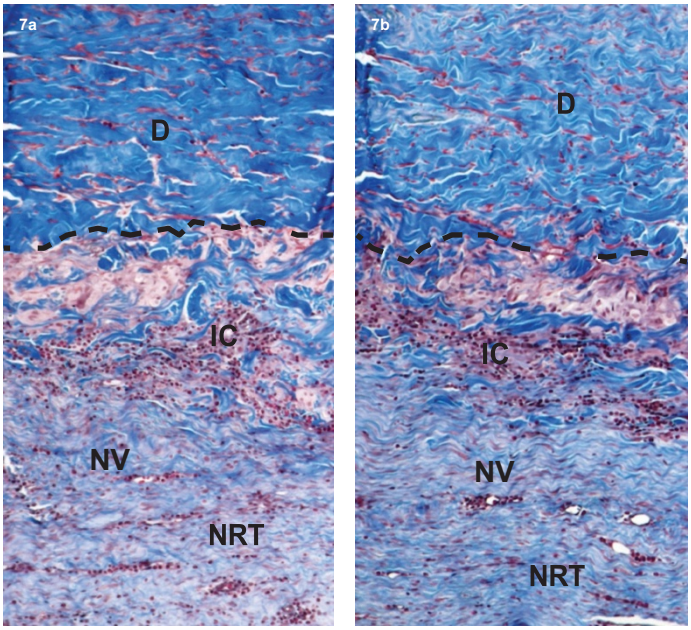

Figure 7. Masson's Trichrome staining at 1 month (10X). Figure 7a: Vivaderm. Figure 7b: Alloderm. Deep cellular infiltration of inflammatory cells (IC) and neovascularization (NV) is observed within newly remodeled tissue (NRT) for both Vivaderm and Alloderm implants (D). Dashed line depicts the remodeling front.

The histological scoring showed favorable results (Table 1). Inflammatory response at this early time point is expected as part of the normal remodeling response and given the xeonogeneic model. Marked new collagen deposition was observed, with three-fourths of the Vivaderm samples scoring moderate to marked collagen formation. No meaningful differences were observed between irradiated and non-irradiated tissue.

This study demonstrated excellent tissue integration with the host and new tissue formation with Vivaderm. Vivaderm's biocompatibility is evident in the rapid cellular infiltration and remodeling of the matrix. No adverse effect of irradiation on the response to the graft was evident. As seen here, implanted matrix clearly plays an active role in enabling normal repair processes.

Table 1. Histological Scoring

	Vivaderm (n=8)	Non-Irradiated ACD (n=8)	Alloderm (n=2)
Inflammatory Cells	1.7	2.1	1.5
Neovascularization	1.9	2.5	1.5
New Collagen Deposition	2.8	3.0	2.0

Note: H&E and Masson's trichrome staining specimens were scored by an independent veterinary pathologist as follows: 0=no response; 1= minimal; 2= mild; 3=moderate; 4=marked or severe. Average histological scores for each parameter are reported.

Conclusion

These three preclinical studies demonstrate that Aziyo's proprietary process of lyophilization and gamma irradiation produces a sterile material that naturally promotes tissue repair *in vivo.* No adverse effects of irradiation were observed in any of the studies.

Vivaderm facilitates all of the stages necessary for healing:

- Normal inflammatory responses needed for tissue integration
- Neovascularization
- Collagen deposition and tissue remodeling

The repair processes begin soon after surgical implantation, with data herein showing neovascularization at 4 days post-transplant. At 30 days, neovascularization is widespread, delivering cells and nutrients that promote new matrix deposition. In allogeneic models by 90 days, inflammatory responses are largely replaced by remodeling indicating successful healing.

Aziyo's exclusive proprietary methodology of terminal sterilization by low dose, low temperature gamma irradiation provides an added margin of safety that may reduce the risk of complications or adverse immune responses to the graft for better patient outcomes.

Russell Health™

Regenerative Medicine is our Specialty

E sales@russellhealth.com **P** 312-919-1896
Russell Health, Inc. | 330 W Diversey Parkway, Suite 1808, Chicago, IL 60657
www.russellhealth.com

Results from laboratory testing or animal studies may not be predictive of clinical experience. Physicians and operating room personnel should review the Package Insert for product information, including indications, contraindications, precautions and warnings.