Does Application of Dehydrated Human Amnion/Chorion Membrane Increase Matrix Metalloproteinase Levels in Wounds?

Thomas Serena, MD
Marissa Carter, PhD
EpiFix VLU Study Group

SerenaGroup™ Wound and Hyperbaric Centers, Cambridge, MA
Strategic Solutions, Inc., Cody, WY

SAWC Fall Meeting, September 26-28, 2015 in Las Vegas, NV
Poster # LB - 016

Abstract

The normal wound healing process occurs in a well-orchestrated and predictable sequence of integrated and overlapping phases including hemostasis, inflammation, proliferation, and tissue remodeling. These interrelated physiological processes create a reparative microenvironment characterized by high initial levels of growth factors and other soluble mediators of cell signaling, controlled levels of proteases and bacteria, and functional fibroblasts, keratinocytes, and vascular endothelial cells. Natural amniotic membrane has been shown to modulate matrix metalloproteinases (MMPs), yet it has been suggested that dehydrated human amnion chorion membrane (dHACM) contains higher levels of MMPs than other amniotic membrane products. As a secondary outcome in a previously reported IRB approved, multicenter, randomized clinical trial comparing incidence of >40% reduction in wound size for venous leg ulcers treated with dHACM allograft or multilayer compression only, swabs were obtained at day 0 and weekly thereafter for the 4 week study period and later analyzed for absolute values of inflammatory proteases (MMP 8 and 9). For this analysis, we included patients with assay values at day 0 (prior to group allocation) and at conclusion of the 4 week study (n=49). MMP results were examined for day 0 and at study conclusion to determine if values had increased, decreased or stayed the same. For those patients receiving dHACM (n=38), MMP values decreased in 15 (39.5%), remained at 0 in 12 (31.6%) and increased in 11 (28.9%). For controls receiving multilayer compression only (n=11), MMP values decreased in 5 (45.4%), remained at 0 in 2 (18.2%) and increased in 4 (36.4%). No significant differences were observed for increased or decreased MMP levels between subjects receiving dHACM and those that did not. These results show that wounds treated with dHACM do not exhibit increased MMP levels.

Background

The normal wound healing process occurs in a well-orchestrated and predictable sequence of integrated and overlapping phases including hemostasis, inflammation, proliferation, and tissue remodeling. These interrelated physiological processes create a reparative microenvironment characterized by high initial levels of growth factors and other soluble mediators of cell signaling, controlled levels of proteases and bacteria, and functional fibroblasts, keratinocytes, and vascular endothelial cells.[1]

Failure to progress through the normal phases of healing in an orderly fashion can result in a chronic wound. Biochemical characteristics of chronic wounds include:

- Elevated inflammatory markers
- High levels of proteases, including matrix metalloproteinases (MMPs)
- Diminished growth factor activity
- Reduced cell numbers in the wound

MMPs play beneficial roles in normal wound healing, but when they are present in the wound bed at increased levels, for too long a period, or in the wrong location in the wound, they can be destructive.[2]

Amniotic membrane in its native form has been shown to modulate MMPs.[3] PURION® Processed dHACM has been shown to retain the growth factors in natural amniotic membrane including PDGF-AA, PDGF-BB, bFGF, TGF-β1, EGF, VEGF, and PlGF.[4] In addition to growth factors, cytokines including anti-inflammatory interleukins (IL-1ra, IL-4, and IL-10) and the TIMPs (TIMP-1, TIMP-2, and TIMP-4) which help regulate the matrix metalloproteinase (MMP) activity are also present in dHACM.[4]

References

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Purpose

To examine MMP levels in chronic venous leg ulcers before and after treatment with dHACM and compare these levels to wounds not treated with dHACM.

Methods

- In a previously reported[5] IRB approved, multicenter, randomized clinical trial comparing incidence of ≥40% reduction in wound size for venous leg ulcers treated with dHACM allograft or multilayer compression only, wound swabs were obtained at day 0 and weekly thereafter for the 4 week study period.
- Absolute values of inflammatory proteases (MMP 8 and 9) were measured.
- For this analysis, we included patients with assay values at day 0 (prior to group allocation) and at conclusion of the 4 week study (n=49).
- MMP results were examined for day 0 and at study conclusion to determine if values had increased, decreased or stayed the same.
- Comparisons between the groups were made using non-parametric statistics as appropriate.

Sample Preparation

- Prior to swabbing, the wound was cleansed with sterile saline solution to remove all loose debris, remains of therapeutic agents, and necrotic tissue; sharp wound debridement was not performed prior to sample collection.
- Complete hemostasis was achieved before obtaining the specimen and areas of necrotic material or thick slough were avoided.
- The wound area to be swabbed was moistened with up to five drops of saline, and the head of the foam swab (Puritan Medical, Guilford, ME, USA) was pressed flat against the base of the wound and gently rotated back and forth several times while applying pressure until it was fully coated with wound fluid.
- The wound fluid sample was then frozen to -70 °C and shipped for laboratory analysis.
- Results of laboratory testing were reported as MMP in U/110uL.

Results

- 49 patients had analyzable wound swabs collected at day 0 and at 4 weeks.
- 38 (77.6%) of patients had received dHACM and 11 (22.4%) received multilayer compression only.
- MMP levels by treatment group are presented on Table 1.
- No significant differences were observed for increased or decreased MMP levels between subjects receiving dHACM and those that did not (Figure 1), or for wounds that were on a trajectory to heal vs. those that were not (Figure 2).

dHACM = EpiFix®, MiMedx Group, Inc., Marietta, GA
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Results

Table 1. Comparison of MMP U/110uL levels.

MMP U/110uL	dHACM (n=38)	Multilayer Compression (n=11)	p-value
Day 0	9.3 ± 31.0 0.15 (0, 155.3)	2.1 ± 5.7 0.08 (0, 19.0)	0.52
Week 4	5.5 ± 14.1 0.005 (0, 79.2)	0.39 ± 0.64 0.13 (0, 1.8)	0.59
p-value	0.96	0.64	

Figure 1. MMP Changes over 4 weeks by Treatment Received

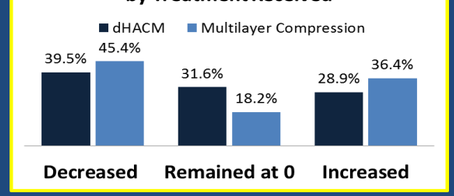
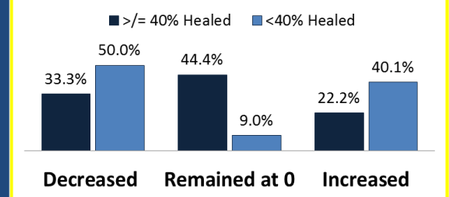


Figure 2. MMP Changes over 4 weeks by Healing Status



Conclusion

- These data suggest that wounds treated with dHACM do not exhibit increased levels of MMPs.
- Further elucidation on the roles of MMPs in the healing of chronic wounds is needed.

EP371.001