USP Testing for Oasis® Wound Matrix

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Statement of Purpose: The management of chronic wounds poses a significant cost for the U.S. healthcare industry. One of the distinguishing characteristics for chronic wounds is a lack of extracellular matrix (ECM) necessary for promoting cellular growth and subsequent wound healing. Oasis® Wound Matrix was developed as a treatment for chronic wounds as a temporary ECM to promote cell growth. Oasis® Wound Matrix is a biologically derived, collagen-based wound care product. It is obtained from the small intestinal submucosa layer of the domestic pig. This layer has been mechanically separated from the adjoining layers of the intestine by removing the serosal, mucosal, and muscular elements. The isolate submucosa is chemically cleaned, decellularized, freeze-dried, and terminally sterilized. Oasis® Wound Matrix consists of about 70 percent protein, 20 percent carbohydrate, and 7 percent lipid by dry weight. The protein component is primarily collagen type I (~90%), with minor amounts of elastin, collagen type III, collagen type IV, and collagen type VI. However, structural proteins alone are not likely enough to promote healing in these chronic wounds. As such, Oasis® Wound Matrix is processed to retain additional extracellular matrix components, including glycosaminoglycans and basic fibroblast growth factor. A United States Pharmacopeia (USP) monograph was created to highlight some specific Oasis® Wound Matrix compositional components and bioactivity. Thirty-six lots of Oasis® Wound Matrix were tested for compliance to the USP monograph.

Methods: Specific guidelines for testing are outlined in the USP monograph. This testing is split up into five separate groups: fibroblast growth factor (FGF-2) content, sulfated glycosaminoglycan (sGAG) content, bioactivity, metabolic activity, and endotoxins. FGF-2 testing was performed on three samples per lot using an ELISA procedure (R&D Systems, Mpls, MN). sGAG content was measured using dye binding assay with colorimetric analysis. To show that the FGF-2 content is biologically active, a bioactivity test measuring percent differentiation of neural cell line was performed. Oasis® Wound Matrix is chemically treated in such a way as to remove all viable cells. Metabolic activity was measured using a dye conversion assay and compared to freshly isolated pig intestine. Endotoxin testing was performed by an LAL assay.

Results/Discussion: The USP monograph sets levels for each of the five categories tested. Fibroblast growth factor content will be > 10,000 pg/g. Glycosaminoglycan content will be >2 µg/mg. Bioactivity, as measured by the percent of differentiated PC-12 cells from Oasis® Wound Matrix samples is >5% and statistically greater than the negative control ($p<0.05$). Metabolic activity reading must have an average reading <0.10. Endotoxin levels should not exceed 20 Endotoxin Units (EU) per 70 cm². The average FGF-2 content was 41808 ± 20747 pg/g and the lowest FGF-2 content was 13775 pg/g. The average sGAG content was 7.27 ± 2.10 µg/mg and the lowest was found to be 3.87 µg/mg. The average bioactivity was 18.8% ± 5% and the lowest value was 10.7%. The average metabolic activity was 0.029 ± 0.038 and the highest reading was 0.073. Endotoxin was below detection limits for all but 3 of the lots tested, the highest level of endotoxins was 0.260 EU. The test standards and results are summarized in the table below. All 36 lots tested passed the USP monograph criteria.

Test	USP Standard	Average	Lowest/ (Highest) Value
FGF-2	>10,000 pg/g	41808 ± 20747 pg/g	13775 pg/g
sGAG	>2 µg/mg	7.27 ± 2.10 µg/mg	3.87 µg/mg
Bioactivity	>5%	18.8% ± 5%	10.7%
Metabolic Activity	<0.10	0.029 ± 0.038	(0.073)
Endotoxin	20 EU/70 cm²	0.200 EU	(0.260 EU)

Conclusions: Oasis® Wound Matrix contains many non-collagenous components (FGF-2, sGAG). These non-structural components likely increase the wound healing promoting capabilities of Oasis® Wound Matrix. Thus, extracellular matrix derived biomaterials that include cellular signals can be effective scaffold for tissue repair. SIS has proven clinically useful for a wide variety of hard to heal chronic wounds. The USP requirements were met for all 36 lots tested.