

## **Dermagraft®: Human Fibroblast-Derived Dermal Substitute**

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Dermagraft is a three-dimensional, scaffold-based, neonatal fibroblast culture that is used in the treatment of chronic wounds. On implantation, cells from Dermagraft are capable of colonizing the wound bed, secreting cytokines, including angiogenic growth factors, and providing a substrate for keratinocyte migration. In addition, they produce inflammatory modulators, such as IL-8, which attract and activate neutrophils to generate antibacterial activity. One possible mechanism of chronic wound formation postulates the failure of the appropriate fibroblast response to injury, resulting from stress-induced senescence in the untreated host. It has been observed that IL-8 secretion decreases in replicatively senescent cells. It is proposed that this causes a failure in antimicrobial activity, allowing bacterial colonization that prevents keratinocyte migration and wound closure.

Dermagraft is an allogeneic cell preparation that does not cause acute immunological rejection. It is made from cells derived from a single individual that are stored as a Master Cell Bank that is extensively tested. The cells are expanded to 5<sup>th</sup> passage in roller bottles, when they are cryopreserved as the Manufacturer's Working Cell Bank, which undergoes further testing. Further testing is performed on fibroblasts grown beyond the final product stage.

The cells are expanded from 5<sup>th</sup> to 8<sup>th</sup> passage (about 28 cell doublings) prior to seeding on a degradable, biocompatible, lactate/glycolate, knitted fabric in flexible (bag) bioreactors. Multiple bioreactors are coupled together in an array as a closed system. The growth process uses a minimum of aseptic operations and maximal automation. Medium, Dulbecco's Minimal Essential Medium supplemented with calf serum, is changed periodically over 15 - 17 days to allow cell proliferation and deposition of cell-secreted extracellular matrix comprising collagen, other matricellular molecules and bound growth factors. At harvest, determined by glucose utilization, individual bioreactors are sealed and cut out of the array to form part of the final packaging. This procedure avoids a final sterile operation or terminal sterilization. The final product is cryopreserved in the presence of dimethyl sulphoxide and stored below -70°C. This operation provides shelf life, which allows time for analytical and microbiological testing (1 month), inventory control. It also induces a stress response, with the induction of several stress response proteins, that may be important for the survival of the cells in the hostile chronic wound environment. The product is distributed at -70° C on dry ice and is thawed by physician. The packaging is designed to facilitate clinical application by the physician.