**In situ Hybridization to Evaluate the Wound Healing Process of a Bilayered Living Cell-Based Construct (Apligraf®) in a Nude Mice Model**

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**Introduction**

Apligraf\(^\circ\) is a bio-engineered living tissue construct comprising human neonatal fibroblasts embedded in a bovine type I collagen gel underlaying a fully differentiated epithelium composed of human neonatal keratinocytes.\(^1\) To investigate remodeling over time, Apligraf was applied to full thickness skin wounds in an athymic nude mice model.\(^2\)

**Materials and Methods**

After 1, 2, 4 and 12 weeks, wound healing was assessed by digital photography and image analysis. At each of these time-points, biopsies were analyzed using histological staining, immunohistochemistry using human-specific antibodies and *in situ* hybridization using in-house constructed RNA probes to examine the expression of the cellular and extracellular matrix components and growth factor(s) such as *coll1a1*, *col3a1* and *vegf-a* respectively.

**Results**

Gross observation revealed no obvious host reaction. Apligraf appeared to be well integrated with host tissue and evidence of vascularization was observed at 1 week as assessed by histology. In contrast to the clinical situation where Apligraf modulates and improves secondary intention wound healing, it behaved like a graft in this athymic mouse model, as evidenced by host cells invading the construct from the edges and human proteins found in the healed / healing wound region after 12 weeks post implantation. Analysis of the gene expression for *coll1a1*, *col3a1* and *vegf-a* via *in situ* hybridization showed that as early as seven days a defined distribution and localization of cells expressing these genes within both implant and host tissue.

**Discussion and Conclusions**

The distribution and localization changed with time according to the wound healing process. Although this method is not quantitative, this approach was useful in observing changes over time in the density of cells expressing these genes. Evaluation of the distribution of the cells expressing *coll1a1*, *col3a1* and *vegf-a* genes over time within the tissue has opened up possibilities to investigate the cross-talk processes at the cellular level involved between the bioengineered living tissue construct and the host.

**References** List references cited in text as


**Acknowledgments**

We thank Aurelie Nallet and Agatha Zawadzka for their technical support.

**Disclosures**

Cecile F. Rousseau and Vincent Ronfard are employees of Organogenesis Inc.. Aurelie Pagnon-Minot was granted by Organogenesis Inc.