

Wound-Care-2020: Study on wound healing after cutaneous lesion and reconstructed Autologous Pigmented Skin Dressing (APSD) in nude mice: GLP-study- Jean Christophe Lepivert- Shankarrao Mohite College, India

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Wound healing is a complex and dynamic process of replacing devitalized and missing cellular structures and tissue layers. The human adult wound healing process can be divided into 3 or 4 distinct phases. Earlier authors referred to 3 phases— inflammatory, fibroblastic, and maturation, which has also been denoted as inflammatory, proliferation, and remodeling—and this is maintained by some authors. In the 4-phases concept, there are the hemostasis phase, the inflammatory phase, the proliferation phase, and the remodeling phase. In the 3-phases approach, the hemostasis phase is contained within the inflammatory phase. Authors develop a process of in vitro skin reconstruction from locally anesthetised patient's biopsies. This process is oriented through applications with patient presenting cutaneous defect as chronic wounds, burn injuries or congenital melanocytic nevus. One step of this development process is reconstructed skin production under Good Laboratory Practices (GLP). Subsequently, application of Autologous Pigmented Skin Dressing (APSD) on immunodeficient mouse model, demonstrates its harmlessness and functionality with required sanitary characteristics. Clinical results will be presented in this paper.

Materials and Methods: This technology consists in reconstructing autologous pigmented skin on a collagen matrix such as IntegraTM or Matriderm[®]. Skin from breast reductions was taken from the operating room and managed to the French Blood Establishment (FBE). Keratinocytes, Melanocytes and fibroblasts were extracted from the biopsy harvested on patient himself and cultured for cells amplifications. On top of the collagen matrix, fibroblasts were seeded to remodel collagen and after this step, keratinocytes and melanocytes were seeded to produce the epidermal layer. APSD were produced in 3-5 weeks. The APSD (Test Item approximately 6 cm²) and its culture media was provided by truck at 18-20°C to testing facility (about 500 km). Testing facility stored under a 37°C, 5% CO₂ humidified atmosphere for up to 24 hours. From the Operating room to mice coverage, skin, cells and reconstructed skin were identified and traceable. From July 2018 to July 2019, 4 groups of 7 mice were implanted. For each group, 6 mice were treated with test item and one or two mice with collagen matrix alone as control. Under general anesthesia defects (3x2 cm) on dorsum of mice was done and covered with APSD or collagen matrix alone. This study was conducted according to GLP and EMEA EMEA/CHMP/410869/2006

1/07/2007) guideline. Wound healing, clinical behavior, any symptom, tumor development, and mortality sign were noticed every day. Weight, food and water consumption were noticed every week.

Results: 1 mouse did not survive to surgery. Groups 1, 3 and 4 healed well. Follow up demonstrated, a good integration of APSD with minor retraction and a diffuse pigmentation. Group 2 healed with multiple millimetric wounds, and during the healing process, skin retraction appeared which increased with weeks. All collagen matrices (control group) didn't heal and made complete skin retraction for skin closure. Except one mouse which had a nice APSD but loss of weight leading to sacrificed, the other mice grew up, drank and ate normally

Discussion: Bioengineered APSD demonstrated enthusiastic results regarding wound healing. Reconstructed skin could be easily handled and shipped far from the reconstructed area. APSD were simply immersed in cultured medium. APSD groups healed well except for one batch for which the quality of cells seeded was bad leading to thin APSD. The electromagnetic field (EMF) has a great impact on our body. It has been successfully used in physiotherapy for the treatment of bone disorders and osteoarthritis, as well as for cartilage regeneration or pain reduction. Recently, EMFs have also been applied in in vitro experiments on cell/stem cell cultures. Stem cells reside in almost all tissues within the human body, where they exhibit various potential. These cells are of great importance because they control homeostasis, regeneration, and healing. Nevertheless, stem cells when become cancer stem cells, may influence the pathological condition.

Conclusion: Next step will be the clinical trial. The first selected patients, which will be treated with the autologous pigmented skin dressing, could have chronic wounds that could not be closed with traditional treatment as patients with bad general condition. Phase 1 could be done in 2020 and time to market calculated for 2025

The integrity of healthy skin plays an important role in maintaining physiological homeostasis of the human body. Many instances are described which lead to insufficient healing necessitating further intervention. Although wound healing mechanisms and specific cell functions in wound repair have been delineated in part, many underlying pathophysiological

processes are still unknown and we are only able to design new and effective wound healing therapies if we better understand this complex interplay. The here presented new perspectives further support the enormous importance of research in this field in order to reduce the incidence of nonhealing wounds and to facilitate the healing process in general.