Developing an Economical Fibrous Gelatin-Polyurethane Scaffold for Skin Regeneration

Mohammadali Sheikholeslam[1,2], Meghan Wright[3], Nan Cheng[1,2], Hwan Hee Oh[1,2], Saeid Amini-Nik[1,2,4]*, Paul Santerre[3,5]*, Marc Jeschke[1,2]*

[1]Sunnybrook Research Institute, [2]Department of Surgery, [3]Institute of Biomaterials & Biomedical Engineering, [4]Department of Laboratory Medicine and Pathobiology, [5]Faculty of Dentistry, University of Toronto, * co-corresponding authors

Statement of Purpose: Wound healing is vital for patients with complex wounds including burns. While the gold standard of skin transplantation ensures a surgical treatment to heal wounds, it has its limitations, including in many cases a limited availability of skin to graft. Current skin substitute technologies remain expensive and require significant clinical management of the skin wounds. Therefore, tissue engineered skin has become a significant strategy of valued interest to the field. Gelatin is an economical, natural biomaterial which is frequently used for tissue engineering applications[1]. However, it suffers from a lack of sufficient mechanical strength, fast degradation. The aim of this study was to conceive and characterize a hybrid acellular scaffold that would inherently promote skin regeneration, while undergoing resorption. It was hypothesized that adding 20% biodegradable polycarbonate polyurethane (PU) [2-3] to gelatin would improve the mechanical properties and degradation rate of electrospun gelatin without alteration in the cell behavior.

Methods: A hybrid gelatin-based electrospun scaffold was fabricated via the use of a different amount of PU, optimized with a mass ratio of gelatin:PU of 80:20 (Gel80-PU20; hereinafter referred to as GPU). It was compared with gelatin (Gel100) and Integra™ (dermal regenerative template, DRT) benchmark scaffold as controls. Physical and mechanical characterization, collagenase degradation and *in vitro* cell viability were performed to evaluate the newly manufactured composite polymer. The scaffolds were transplanted onto the full thickness excisional wounds of mice and kept for 20 days. The mice were euthanized, and the wounds were excised along with 2 mm of satellite skin for further histological evaluation. All procedures comply with the guidelines of Welfare Committee of the University of Toronto. Masson's trichrome staining was performed to assess cell infiltration from the wound bed and scaffold degradation *in vivo*. Immuno-fluorescence (IF) staining and immuno-histochemistry (IHC) were performed to evaluate the extent of macrophage absorption of grafted scaffold, myofibroblast and new tissue formation. Biosafety of the scaffolds has further evaluated in pigs for 30 days.

Results: The results showed that introducing 20% of a degradable and cell compatible PU to gelatin does not change the fiber and pore size of an electrospun gelatin scaffold while both the elongation and tensile strength were significantly increased even better than commercial DRT. It was also observed that the scaffold has a better elasticity than DRT (Fig. 1a-c). On the other hand, adding 20% PU to gelatin improved degradation resistance in collagenase solution from few hours to 14 days (Fig. 1d). This is important as early degradation of the scaffold adversely affects its ability for providing a framework for cell infiltration and skin regeneration. Live/Dead assay showed more than 90% cell viability in all three scaffolds when human dermal fibroblast (HDF) was incorporated onto the scaffold. This showed that presence of PU does not adversely affect cell viability on the gelatin scaffolds. Due to the limitations of the Gel100, revealed during the characterization part, only GPU and the DRT were investigated *in vivo*. Trichrome staining revealed that both scaffolds allow cell infiltration into the scaffold from the wound bed and as the cells infiltrate, they degrade the scaffold and deposit new ECM. Both of the scaffolds promoted collagen deposition and vascularization that are necessary for a successful skin substitute. However, the electrospun scaffold was more populated with cells. IF showed less macrophages in GPU (Fig. 1e) which indicated that the scaffold does not elicit (or at least shows a milder) foreign body response or chronic inflammation after transplantation. Also, less myofibroblasts were observed on the GPU scaffold. This can be beneficial for preventing wound contraction and fibrosis in favor of skin regeneration[4]. Grafting the scaffold into the large skin biopsy of the pigs showed that GPU is safe, degrades totally over 30 days and lead to new dermis formation which is comparable with the commercial DRT (Fig. 1f).

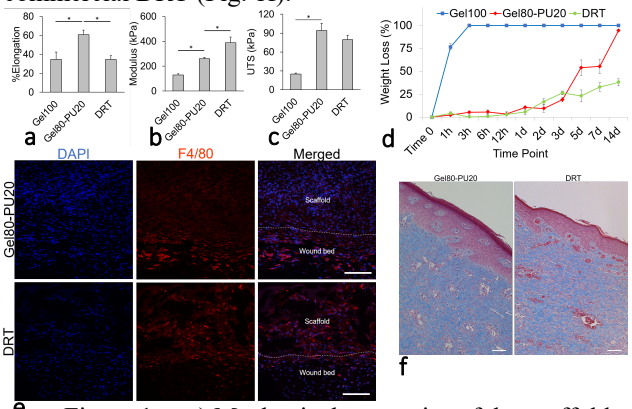

Figure 1. a-c) Mechanical properties of the scaffolds. d) Collagenase degradation of the scaffolds e) Immunostaining of the mice wounds (covered with scaffolds for 20 days) for a macrophage marker (F4/80). Trichrome staining of the pig wounds (covered with scaffolds for 30 days).

Conclusions: The findings show that electrospun GPU scaffolds hold very good potential as substitutes for current commercial DRT systems, which still have limitations in wound care. Presented data persuades extended experiments to be done on preclinical animals such as pigs.

References:

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