Optimization of Tissue-Engineered Skin Produced in vitro: Impact of Fabrication Method and Air-Liquid Interface Maturation on the Contractile Behavior

Robert Gauvin1,2,3, Danielle Larouche1,2, Rina Guignard1,2, Hugo Marcoux1,2, François Auger1,2, Lucie Germain1,2.
1 LOEX, Hôpital du Saint-Sacrement, Centre Hospitalier Affilié Universitaire de Québec (CHA), Québec, QC., Canada.
2 Department of Surgery, Université Laval, Québec, QC., Canada.
3 Currently at the Harvard-MIT Division of Health Sciences and Technology (HST), Cambridge, MA, USA.

Introduction
There is a clinical need for the development of tissue-engineered skin showing minimal contraction at the time of implantation for applications requiring permanent skin replacement. Our group has developed a method to produce engineered tissues without any exogenous scaffold: the self-assembly approach. This method is based on the capacity of cells to secrete and assemble extracellular matrix proteins and allow for the fabrication of a fully autologous tissue-engineered skin (TES) comprising a dermis and a differentiated and stratified epidermis, resulting from air-liquid interface maturation. The present study was designed to evaluate the impact of fabrication method and the presence of the epidermis on the contractile behavior of the final tissue-engineered constructs.

Materials and Methods
Our method for producing a tissue-engineered skin (TES) based exclusively on the use of human cells without artificial scaffolding has previously been described [1]. Briefly, dermal fibroblasts (DF) were cultured in presence of ascorbate until the formation of a cohesive tissue sheet. Three tissue sheets were superimposed and were cultured 7 days to allow for the sheets to adhere to one another and to form a tissue-engineered dermis (TED). Following the production of the TED, keratinocytes (K) were seeded on top of the TED and were maintained in submerged culture condition until they form a confluent layer. The engineered tissue was then cultured at the air-liquid interface using a custom built air-liquid culture support, to initiate K differentiation and to promote the formation of a stratified epidermis. Different assembly methods were developed to optimize the fabrication process and the mechanical integrity of the TES. Long-term contraction measurements were performed in order to analyze the influence of assembly methods, keratinocytes and maturation at the air-liquid interface on the stability of the engineered tissues. Moreover, mathematical modeling of contraction kinetics was performed to provide an easy comparison between the contractile behaviors of the various skin substitutes.

Results
The different assembly methods used in the present study did not make noticeable differences on the histological aspect of the engineered tissues, every method showing a well differentiated and stratified epithelium following air-liquid interface maturation. Contraction measurements showed that all tissue tested displayed a characteristic exponential decay profile, independently of the assembly method and culture conditions. TED contracted significantly faster when compared to the other conditions. The addition of K on the TED followed by submerged culture slowed, but did not prevent the contraction phenomenon, whereas contraction was significantly reduced in differentiated skin substitutes cultured at the air-liquid interface. Modelisation of the contractile behavior was achieved using an algorithm allowing for the determination of the decay amplitude (C), the rate constant (α) and the baseline offset (γ), characterizing the contraction kinetics of the different tissues studied. These parameters were used to establish statistical significance between the measured contraction for the different assembly methods and culture steps involved in the production of the engineered tissues. Results showed that the presence of a differentiated epidermis significantly reduce the amount of contraction experienced by the engineered tissues, independent of the assembly method used for their production (p<0.05).

Discussion and Conclusions
The dermal portion of the skin is known to participate to the contractile behavior of skin grafts [2,3]. The present study demonstrates that the addition of keratinocytes and the formation of a differentiated epidermis, resulting from maturation at the air-liquid interface, stabilizes the structure of the self-assembled TES and reduces greatly its contraction, potentially improving its clinical performance for wound treatment. These results have a considerable impact since the clinical use of skin substitutes requires a tissue with low contraction to restore skin functionality and ensure adequate wound healing.

References

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