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Optimization of the Potential of Human Adult and Embryonic Stem Cells for Skin Tissue Engineering

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ABSTRACT
Current treatments for massive skin loss are still in their infancy and Skin Tissue Engineering (TE) remains as the most promising approach to produce a universal skin substitute that would provide burn victims and other patients the best quality skin in the shortest time.
Despite having the longest history of commercialization, skin analogues lack in meeting completely the regeneration demands [1]. In fact, the long-term function of the skin equivalents could be limited, among other factors, by the terminal differentiation of the grafted keratinocytes. The main ambition of this work-plan is to develop novel skin Tissue-engineered constructs that stimulate regeneration rather than dressing of full-thickness skin wounds.
Stem Cells have emerged as a powerful tool for treatment of a wide range of diseases, providing an exclusive unlimited source of biological material namely for Skin TE. The potential of human Mesenchymal Stem Cells (hMSCs) and Embryonic Stem Cells (hESCs) for skin regeneration has been herein studied, aiming at guarantying an active source of biological material, crucial for full-thickness skin defects and towards a clinical application.
Adult MSCs integrate several mammalian tissues and although their primary function is homeostasis maintenance, they also seem to still express pluripotency markers, and appear to participate in the regeneration of other tissues besides mesenchymal lineages [2, 3]. Human adipose-derived stem cells (hASCs) in particular, are highly attractive due to their abundance and readily accessibility to subcutaneous adipose tissue and to the features shared with other MSCs. Besides that, hASCs can be used in autologous approaches, are easy to expand *in vitro* and present a great differentiation potential, including towards the endothelial and neuronal lineages, generated from the same germ layer as epidermis [4].
hESCs, derived from the inner cell mass of pre-implantation embryos at blastocyst stage, are natural favourite candidates for obtaining the main skin cell types. The most obvious factors rely on their immortality capacity, self-renewal *ad* infinitum and differentiation into all components of the embryonic germ layers and subsequently all cell types that comprise human tissues [5]. Thus, hESCs can potentially provide an extraordinary source of cells for TE together with a great insight into early embryonic development.
As efficient approaches towards relevant skin cell lineages are still to be concisely defined, this is a major issue that has been addressed in the present PhD. Thus, different strategies, based mainly in co-culture systems with adult cells, including indirect (Conditioned media and transwells®) and direct contact approaches, have been defined to differentiate stem cells into key skin cells, keratinocytes (Fig1) and endothelial cells.

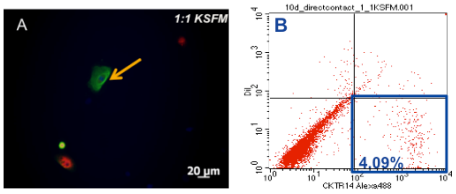

Fig 1. Directing hASCs towards epidermal lineage by direct contact. (A) Immunocytochemistry and (B) Flow cytometry analysis of early epidermal marker cytokeratin14, after 5 and 10 days of contact, respectively.

Furthermore, as skin stem cells namely Epidermal Stem Cells (EpSCs) are a naturally privileged sub-population in the skin tissue,

strategies to promote enrichment of this fraction are being currently undertaken [6]. This comprehends extensive characterisation and employment of substrata/chemical stimuli, (Fig.2) along with selective methods (Fig3).

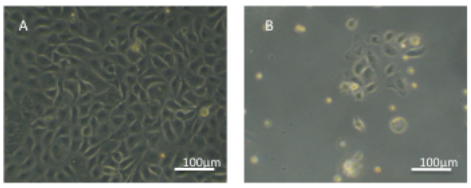

Fig 2. hKC (A) after rapid adherence to Col. IV, and (B) without treatment

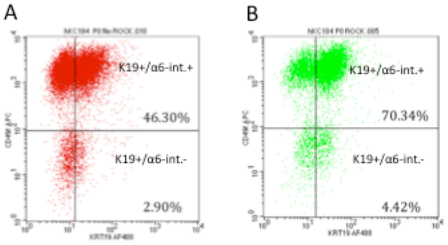

Fig. 3 Rock Inhibitor treatment effect in hKC, fractions (A) without Rock treatment and (B) with Rock treatment were analysed for K19/α6 integrin expression, a critical surface marker combination to identify EpSCs, by Flow Cytometry.

These approaches allow the identification of the most promising source and methodology to obtain stem cells sub-populations to be further combined with polymeric scaffolds in novel Skin TE constructs.

Ultimately, the central goal is to establish the most appropriate cell niche for skin TE approaches, using the exploited cell sources. *Gellan gum*, a thermoresponsive hydrogel (Fig.4A), is herein proposed to mimic dermis mechanical features.

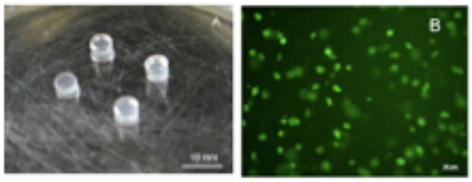

Fig. 4 (A) Thermoresponsive hydrogel, Gellan Gum. (B) Human Dermal Fibroblasts encapsulated in gellan gum membranes, after 3 days of culture, labelled with Calcein-AM, that stains live cells.

In addition to the obtained stem-derived skin cells, EpSCs and adult cells from human skin will be assembled with gellan-gum to form a skin tissue engineered construct (Fig.4B) aiming at treating full-thickness wounds.

The suitability of the proposed strategies will be assessed *in vitro* and validated *in vivo* by evaluating the regeneration efficiency in a mouse full-thickness model. Ultimately, it will be possible to compare the potential of committed skin cells, stem-derived cells and pre-committed cells, the EpSCs.

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AUTHOR BIOGRAPHY

Mariana Cerqueira was born in 1983 in Porto, Portugal, where in 2006 concluded her BSc in Biology, in the Faculty of Sciences of the University of Porto. She is currently in her third year of PhD in *Tissue Engineering, Regenerative Medicine and Stem Cells* under supervision of Prof. Rui Reis, at 3B's Research Group. Part of her PhD project was carried out between June-09 and February-10, at *Stem Cell Laboratory*, King's College London, UK under co-supervision of Dr. Stephen Minger.