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Using human umbilical cord cells for tissue engineering: A comparison with skin cells

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ABSTRACT

The epithelial cells and Wharton's jelly cells (WJC) from the human umbilical cord have yet to be extensively studied in respect to their capacity to generate tissue-engineered substitutes for clinical applications. Our reconstruction strategy, based on the self-assembly approach of tissue engineering, allows the production of various types of living human tissues such as skin and cornea from a wide range of cell types originating from post-natal tissue sources. Here we placed epithelial cells and WJC from the umbilical cord in the context of a reconstructed skin substitute in combination with skin keratinocytes and fibroblasts. We compared the ability of the epithelial cells from both sources to generate a stratified, differentiated skin-like epithelium upon exposure to air when cultured on the two stromal cell types. Conversely, the ability of the WJC to behave as dermal fibroblasts, producing extracellular matrix and supporting the formation of a differentiated epithelium for both types of epithelial cells, was also investigated. Of the four types of constructs produced, the combination of WJC and keratinocytes was the most similar to skin engineered from dermal fibroblasts and keratinocytes. When cultured on dermal fibroblasts, the cord epithelial cells were able to differentiate in vitro into a stratified multilayered epithelium expressing molecules characteristic of keratinocyte differentiation after exposure to air, and maintaining the expression of keratins K18 and K19, typical of the umbilical cord epithelium. WJC were able to support the growth and differentiation of keratinocytes, especially at the early stages of air–liquid culture. In contrast, cord epithelial cells cultured on WJC did not form a differentiated epidermis when exposed to air. These results support the premise that the tissue from which cells originate can largely affect the properties and homoeostasis of reconstructed substitutes featuring both epithelial and stromal compartments.

1. Introduction

The development of reconstructed living substitutes has greatly progressed in recent years, in particular in the field of skin reconstruction, which is among the most advanced for tissue engineering applications. Achieving tissue homoeostasis within reconstructed substitutes is especially important for self-renewing tissues such as skin, which features a differentiated epithelial compartment influenced by the underlying dermal compartment (McLoughlin, 1963). Tissue-engineered substitutes generated using the self-assembly method are useful tools for gaining insights into cell interactions and the regulation of tissue homoeostasis. Indeed, being by nature made of cells and the native extracellular matrix they secrete and organise upon stimulation with ascorbic acid, these living tissues recreate a highly physiological tissue context

Abbreviations: DF, Dermal fibroblasts; DMEM, Dulbecco–Vogt modified Eagle's medium; EDTA, Ethylenediaminetetraacetic acid; EGF, Epidermal growth factor; K, Keratinocytes; K10, Keratin 10; NCS, Newborn calf serum; P, Passage; SEM, Standard error of the mean; TRITC, Tetramethylrhodamine-5-(and-6)-isothiocyanate; UC, Umbilical cord; ucEpi, Umbilical cord epithelial cells; WJC, Wharton's jelly cells

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(Paquet et al., 2010). Such substitutes are particularly helpful for understanding the contribution of stromal–epithelial interactions to differentiation patterns and regulation (Trottier et al., 2008; Carrier et al., 2009) in addition to being employed in the treatment of skin defects (Boa et al., 2013).

Umbilical cord-derived cells have spurred increasing interest in recent years because of their advantageous characteristics, such as a less differentiated status possibly contributing to lower immunogenicity (Ennis et al., 2008; Weiss et al., 2008). In addition to cord blood stem cells, cells derived from the umbilical cord solid tissue are of great interest. Of the four cell types that can be successfully extracted and cultured from a single umbilical cord (Hayward et al., 2013), endothelial cells and smooth muscle cells of the vein and arteries have been extensively used in various experimental settings (Jaffe et al., 1973; Owens, 1995; Bachetti and Morbidelli, 2000), including tissue engineering (L'Heureux et al., 1998; Stephan et al., 2006; Gibot et al., 2010; Guillemette et al., 2010; Rochon et al., 2010; Tsigkou et al., 2010 Gauvin et al., 2011). The Wharton's jelly cells (WJC) have also become the subject of intensive research in recent years (reviewed in Marcus and Woodbury (2008), and in Troyer and Weis, (2008)) with an ever-increasing number of studies demonstrating their characteristics as multipotent stem cells. The epithelial cells of the umbilical cord show an interesting pattern of keratin expression, combining characteristics of simple and stratified epithelia (Mizoguchi et al., 2000; Hayward et al., 2013). Recent studies have highlighted their similarity to neonatal keratinocytes in the pattern of K1/K10 and deltaNp63 expression (Ruetze et al., 2008). They are also able to form a stratified epithelium when seeded on collagen gels populated with fibroblasts (Mizoguchi et al., 2004; Sanmano et al., 2005; Ng et al., 2008; Huang et al., 2011). In this study, we evaluated their behaviour in combination with WJC or dermal fibroblasts in the context of a tissue-engineered skin substitute.

The aim of the present study was to evaluate the impact of epithelial and stromal cells from two different cell sources on the tissue production of bilayered substitutes by the self-assembly approach of tissue engineering. Our results show that the different combinations of skin- and umbilical cord-derived cells had a great impact on resulting differentiation pattern of the epithelial compartment as well as on the development of a mature dermo-epidermal junction. Umbilical cord epithelial cells cultured on dermal fibroblasts differentiated in a similar fashion to keratinocytes when exposed to air, showing the presence of molecular markers characteristic of stratified, keratinised epithelia. Keratinocytes on WJC also differentiated in a similar fashion, but umbilical cord epithelial cells on WJC did not produce a multi-stratified, skin-like epithelium in air-exposed culture conditions. Thus, not all cell sources are interchangeable, and the particular combination of cells can have as much impact on the resulting tissue as culture conditions.

2. Materials and methods

2.1. Cell extraction and culture

Human umbilical cords ($N=6$ different donors) were obtained from the obstetrics unit of the Saint-Sacrement Hospital after normal births, with informed consent from the mothers. All protocols were approved by the Research Ethical Committee of the Centre hospitalier *affilié* universitaire de Québec (CH*A*). Keratinocytes were obtained from newborn foreskin as previously described (Germain et al., 1993), and epithelial cells were extracted from the human umbilical cord using a solution of 0.25% trypsin (ICN) with 2.2 mM EDTA in buffered saline solution, as described in Hayward et al. (2013). For passaging, these cells were seeded in 75 cm^2 culture flasks at 1×10^6 cells per flask with a feeder layer of irradiated Swiss 3T3 cells (2×10^4 cells/cm^2; Germain et al., 1993) and cultured as described below. Dermal fibroblasts were obtained from an adult skin biopsy (23-year-old female donor) as in a prior description (Auger et al., 1995). Umbilical cord WJC were isolated as described in Hayward et al. (2013). Both types of stromal cells were seeded in 75 cm^2 culture flasks at 1×10^4 cells/cm^2 and cultured as described below. All cell populations were cryopreserved in liquid nitrogen pending their use in the experiments, then thawed and cultured for at least one passage. All cell cultures and reconstructed tissues were maintained at 37 °C in a humidified atmosphere containing 8% CO_2, and media were changed three times per week.

Keratinocytes and epithelial cells were cultured in Dulbecco–Vogt modified Eagle's medium (DMEM) combined with Ham's F12 medium (both Gibco BRL, Burlington, Canada) in a 3:1 proportion (referred to as DME–Ham's), supplemented with 10% newborn calf serum (NCS; Fetal Clone II, HyClone, Aurora, Canada), epithelial additives [24.3 μg/mL adenine (Chiron Corp., Emeryville, U.S.A.), 10 ng/mL epidermal growth factor (EGF; Austral Biological, San Ramon, U.S.A.), 5 μg/mL insulin (Sigma Chemical Co., St. Louis, U.S.A.), 0.1 nM cholera toxin (ICN Biomedical, Montréal, Canada), 5 μg/mL transferrin (Boehringer Mannheim, Laval, Canada), 2 nM 3,3′,5 triiodo-L-thyronine (Sigma), 0.4 μg/mL hydrocortisone (Calbiochem, La Jolla, U.S.A.)], and antibiotics [100 IU/mL penicillin G (Sigma), 25 μg/mL gentamicin sulphate (Schering, Pointe-Claire, Canada)]. Fibroblasts and WJC were cultured in DMEM (Gibco BRL), supplemented with 10% foetal calf serum (HyClone) and antibiotics.

2.2. Reconstructed bilayered stromal/epithelial substitutes

Four different reconstructed substitutes were produced in this study, using the following combinations (Table 1): dermal fibroblasts with skin keratinocytes (DF/K), dermal fibroblasts with cord epithelial cells (DF/ucEpi), WJC with keratinocytes (WJC/K) and WJC with cord epithelial cells (WJC/ucEpi). For each combination of specific cell populations, up to seven substitutes were prepared, which allowed sampling of two substitutes for final analysis at each time point. A total of six umbilical cord cell populations (three epithelial and three stromal) were used in five different epithelial cell–WJC combinations, along with one dermal fibroblast cell population and one keratinocyte cell population for comparison purposes.

Bilayered stromal/epithelial substitutes comprising both an epidermal and a stromal layer were prepared using the self-assembly approach of tissue engineering as previously described (Michel et al., 1999). Briefly, dermal fibroblasts or WJC between P3 and P7 were seeded in a 25 cm^2 culture flasks at a density of 2×10^5 cells per flask with 50 μg/mL sodium L-ascorbate (Sigma) in the culture medium, which promotes the deposition of extracellular matrix material. Fresh ascorbic acid was added at each

Table 1
Composition of the bilayered stromal/epithelial substitutes.

DF/K	DF/ucEpi	WJC/K	WJC/ucEpi
Keratinocytes (K)	UC epithelial cells (ucEpi)	Keratinocytes (K)	UC epithelial cells (ucEpi)
Dermal fibroblasts (DF)		Wharton's Jelly Cells (WJC)	