



EUROPEAN ASSOCIATION OF TISSUE AND CELL BANKS

Position of the EATCB on the classification of noncultured autologous epidermal cell (keratinocytes and melanocytes) suspensions

1. Keratinocytes and melanocytes

The epidermis is the outermost skin layer of ectodermal origin. It is composed of keratinocytes (the major cell type in the epidermis) and melanocytes, but also contains Langerhans cells, Merkel cells and inflammatory cells. Keratinocytes are highly specialized epithelial cells designed to perform separation of the organism from its environment.

Melanocytes are located between the basal layer cells of the epidermis and communicate, through dendritic processes, with about 30-40 keratinocytes in what is called the epidermal melanin unit. Melanocytes synthesize melanins in melanosomes, which are transported into keratinocytes to protect them from UV radiation.

2. Treatment of skin lesions or diseases using skin cell and tissue grafts

The clinical use of cultured epidermis was introduced in the early 1980s to treat burns (Pittelkow & Scott, 1986) and later extended to the treatment of many other pathologies, including vitiligo (Pianigiani *et al.*, 2005). The first clinical study was performed in 1987 on a patient with piebaldism (Lerner *et al.*, 1987). The aim of the melanocyte graft is to repopulate achromic areas with functional cells taken from normally pigmented areas. Several procedures have been devised and tested, varying from the simple transfer of epidermis sampled and implanted as is, to the transplantation of disaggregated cells.

The transfer of cultured (Falabella *et al.* 1992) or non-cultured disaggregated autologous epidermal cells (Speeckaert & van Geel, 2017) has been demonstrated to restore the epidermal-melanocyte unit function in pigmentary disorders (e.g. vitiligo and piebaldism).

Epidermal cell suspensions were shown to be equally effective as epidermal tissue grafts and, in addition, allowed for the treatment of larger depigmented areas (Ezzedine *et al.*, 2015).

The application of non-cultured epidermal cell suspension (melanocytes and keratinocytes) has been used and documented by different groups (Ebrahimi *et al.*, 2015; Ramos *et al.* 2013). According to Mulekar (2004 and 2016), this method represents a middle ground between skin grafts and cultured cell transplantation. Epidermal cell suspensions have also been used to treat chronic wounds (Zhao *et al.*, 2016).



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A recent technique based on aerosol spraying of non-cultured epidermal cell suspensions represents an efficacious and rapid way to obtain re-epithelialisation and to treat a variety of epidermal defects such as burns and traumatic injuries, but also in scar reconstruction, donor-site repair and in skin resurfacing techniques (Navarro *et al.*, 2000; Mcheik *et al.*, 2014).

3. Processing of non-cultured epidermal cell suspensions

Basically, enzymatic digestion is used to obtain autologous epidermal cell suspension. This process usually consists of the incubation of small pieces of pigmented skin in a dispase or trypsin suspension (typically 0,25% trypsin in 0,08% wt/vol EDTA) for 30 minutes at 37°C. The mixture is subsequently vortex mixed to separate the epidermis from the dermis and to obtain dissociation of the epidermal cells. The dermis is removed and the resulting suspension containing the epidermal component is centrifuged to achieve a cell pellet, which is resuspended in a predefined amount of saline solution. The resulting epidermal cell suspension, consisting of all epidermal cell types is transferred to the patient.

These epidermal cell suspensions

- are not expanded by cell cultivation
- are not administered on or in a scaffold
- are administered as a suspension
- maintain their biological characteristics
- keep their physiological functions
- restore or form original tissue structures upon application
- are intended to be used for the same (before and after transplantation) essential function in the patient
- are not industrially processed (basically enzymatic treatment)

4. Reasons to regulate noncultured autologous epidermal cell under Tissues and Cell legal framework

4.1. Scientific considerations

There is extensive experience with this procedure, with the implementation of EU Directives on T & C the activity was performed under laminar flow hood in a



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processing room. No major complication, adverse event or reaction was observed

Enzymatic digestion is not specifically included in the list that enumerates what should not be considered as a substantial manipulation, but it is a form of cell separation, an activity that *is* indeed listed as a non-substantial manipulation.

There is scientific evidence that the protocols used to obtain non-cultured epidermal cells, including dispase and trypsin treatment, do not affect the epidermal cell characteristics. For example, the keratinocytes' ability to attach and promote renewal of epidermis and the melanocytes' ability to attach and induce pigmentation are not altered. (Cervelli *et al.* 2009)

Interesting studies have revealed that, in suspension, keratinocytes produce junctional proteins, including desmosomes, markers of suprabasal, differentiating epidermal layers (Yin *et al.*, 2004). Keratinocytes in suspension clump into clusters and – if seeded onto viable physiological substrates - are able to adhere, proliferate and stratify into multiple layers (Pianigiani *et al.*, 2010).

Considering these unchanged biological characteristics and physiological functions of the epidermal cells, the manipulation level should be considered as non-substantial. If the enzymatic digestion leads to isolation of functionally intact tissue units (e.g. pancreatic islets) or there is scientific evidence that the original structural and functional characteristics are maintained, the procedure is not considered substantial manipulation.

According to *Reflection paper on classification of advanced therapy medicinal products 2015*, the “same essential function for a cell population” means that the cells when removed from their original environment in the human body are used to maintain the original functions in the same histological environment.

Non-cultured epidermal cells used for the treatment of vitiligo and other types of leukoderma are returned and integrated back to the same histological site where they keep on functioning according to their initial characteristics. They used for the same essential function, before and after transplantation, as the goal of the transplantation is to transfer melanocytes from a pigmented donor site to an acceptor site that is lacking melanocytes.

In the treatment of burn wounds, epidermal cells also maintain their biological characteristics as they promote the proliferation of epidermal cells (and thus re-epithelization). They help regain or reform original skin tissue structures after transplantation (De Corte *et al.*, 2012), to enhance closure of the wound. Early wound closure reduces excessive collagen production by fibroblast, thus will finally result in a better cosmetic outcome of the scar (Sood R *et al* 2015)



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4.2. Regulatory considerations

During many years TEs processed noncultured autologous epidermal cells under the umbrella of T&C regulations with good (documented) outcomes and without safety or quality issues. The safety and quality requirements under Directive 2004/23/EC therefore seem to be adequate.

4.3. Access consideration

From the economic point of view, there are only few patients, so there is a very small 'market'. This reflects in limited commercial interest and limited intention of pharmaceutical companies to bring this therapy to the market. A pharmaceutical pathway would also require a need to overcome very high reimbursement levels. EACTB therefore believes that access to the treatment can be better if it is regulated under the Human Tissue & Cell legislation. Nevertheless there is no harmonized view within the different Competent Authorities to classify noncultured autologous epidermal cell suspension either as ATMPs or to not (in the latter case they would be governed solely by the Human Tissue & Cell legislation) and this situation creates confusion within the field and more specifically within the Tissue Establishments.

5. Conclusions

EATCB and the panel of consulted experts consider that non-cultured autologous suspensions of epidermal cells fulfil the criteria to be adequately regulated by Human Tissue and cell legislation, based on the following arguments: and this because:

- There is extensive experience using the T&C legal framework with good (documented) outcomes, and without any serious adverse event or reaction. This demonstrates that the quality and safety criteria included in the EU TC Directive are appropriate cover this procedure
- Are basically obtained by cell separation through enzymatic digestion, which is an activity that *is* listed as a non-substantial manipulation according to Regulation (EC) No 1394/2007,
- Biological characteristics, physiological functions or structural properties relevant for the intended regeneration, repair or replacement remain unaltered.
- *Are* intended to be used for the same essential functions in the acceptor site (vitiligo) as in the donor site (pigmented skin) or in the case of burn wounds



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- Are *not* prepared industrially or manufactured by a method involving an industrial process, and are *not* placed on the market in EU Member States, and therefore fall outside the pharmaceutical framework

6. Experts that contributed to this document

- Annika Hakamäki, PhD, Tissue Establishment Responsible Person, Regea Cell and Tissue Center, Faculty of Medicine and Health Technology, Tampere University, Finland
- Cornelia D. Richters, PhD, Tissue establishment Responsible Person ex art 17, 23/2004/EC, Stichting ETB-BISLIFE, Leiden, The Netherlands
- Elisa Pianigiani, MD, Head of Siena Skin Bank, Dept. of Dermatology, Le Scotte University Hospital, Siena, Italy.
- Jean-Paul Pirnay, PhD, Head of the Laboratory for Molecular and Cellular technology (harboring licensed human cell and tissue banks), Queen Astrid Military Hospital, Brussels, Belgium.
- Jürgen Ehlers, General Manager, German Institute for Cell and Tissue Replacement DIZG, Berlin, Germany
- Matthias Löwel, PhD, Head of Regulatory Affairs – Quality Assurance, *German* Institute for Cell and Tissue Replacement DIZG, Berlin, Germany
- Jacinto Sánchez Ibáñez, MD. President of the EATCB. Responsible of the Tissue Establishment. A Coruña University Hospital. Spain



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Jacinto Sánchez Ibáñez.
President of the European Association of Tissue and Cell Banks