



Document	Recommendations for Stem Cell Culture (RSCC)
Revision	0
Page	1 of 2
Operative from	01/02/2012

EEBA RECOMMENDATIONS FOR STEM CELL CULTURE*

Treatment of severe limbal stem cell deficiency can currently include transplantation of cultured stem cells.

Culture of stem cells is a process which requires trained personnel. The process should be performed in a grade A environment.

Patients receiving transplantation of cultured stem cells should be included in a controlled prospective clinical trial because the scientific background on this new technology is currently insufficient for considering it as a routine procedure.

Suitably trained personnel should perform all tasks according to validated, up-to-date, document-controlled standard operating procedures (SOPs) during:

- Tissue or cell retrieval
- Cell processing and culture
- Cultured cell selection

The possible sources of cells for culture currently include autologous limbal explants, autologous oral mucosal explants, and allogeneic limbal explants. From current scientific background, autologous limbal explants are currently considered efficient and acceptable for growing limbal epithelial stem cells for transplantation. Scientific background on other sources of cells is not yet enough to draw conclusions.

Selection and screening of donor tissue (source of explants) should be performed according to a standard operating procedure.

Cells can be cultured directly from explants or from dissociated cells obtained by enzymatic dissociation of explant epithelium. From current scientific background, the limbal explant culture system is considered efficient and acceptable for growing limbal epithelial cells for transplantation (but with high risk of fibroblast contamination). Dissociating cells enzymatically is considered efficient and acceptable for growing epithelial cells. Dispase and EDTA are considered efficient and acceptable for dissociating epithelial cells for culture.

Composition and preparation of the medium used for growing cells should be described in a standard operating procedure. Among possible medium components, DMEM and Ham F12 media, cholera toxin, transferring, adenine, hydrocortisone, L-glutamine, penicillin, amphotericin B, and Epidermal Growth Factor are considered efficient and acceptable for growing cells.

* By the EEBA Stem Cell Cultures Special Interest Group: V. Borderie (Chair, Paris), S. Ferrari (Venice), F. Mayo (Lausanne), D. Meller (Essen), P. Eberwein (Freiburg), O. Damour (Lyon), J. Daniels (London). Recommendations to be reviewed annually. Next revision: Jan 2013



Document	Recommendations for Stem Cell Culture (RSCC)
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Page	2 of 2
Operative from	01/02/2012

Feeder cells are used by several labs with no reported side effects. However, evidence of safety for the recipient has still to be demonstrated.

Possible substrate/carriers for culture include human amniotic membrane, fibrin, and temperature-responsive plastic. Among these substrates, fibrin gel is considered efficient and acceptable.

Cultured cells should be evaluated for microbiological contamination. Microbiological techniques used for controlling cultured cells, samples, and time for taking samples should be described in a standard operating procedure. Bacterial culture is considered efficient and acceptable for assessing the cell culture product. Viral PCR is considered efficient and acceptable for assessing the cell culture product. Microbiological assessment should be performed on feeder cells and culture medium obtained during cell culture, before and during culture.

The cell culture product should be assessed according to a standard operating procedure. Phase contrast microscopy considered is efficient and acceptable for assessing morphology of cultured cells. Immunofluorescence is considered acceptable for assessing cell phenotype.