

# Fibroblast cell therapy

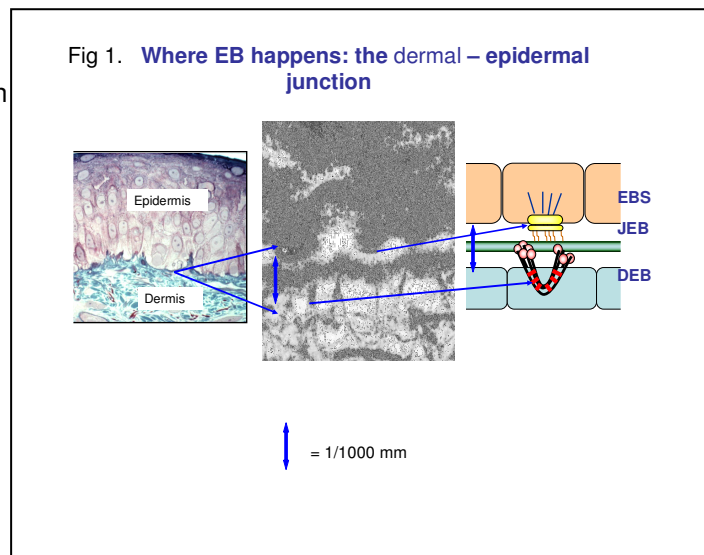
Fibroblast cell therapy is one of several technologies currently in development to attempt to deliver treatments, and ultimately, we hope, cures, for the various forms of Epidermolysis bullosa (EB). EB is a group of heritable skin-blistering conditions that are painful, disabling, disfiguring and, depending on the EB type, frequently fatal, either in infancy or young adulthood.



## What is EB?

The genetic errors in EB result in defects in the proteins at the dermal-epidermal junction (DEJ) responsible for adhesion of the epidermis and dermis (Fig 1). EB forms are broadly classified by the plane of cleavage between the dermis and epidermis into three main types: EB Simplex (EBS), Junctional EB (JEB), and Dystrophic EB (DEB), although around 25 subtypes of EB within these 3 types are recognized.

Over the past 15-20 years, 10 major genes responsible for the majority of cases of EB have been identified, with defects in keratins 5 and 14, and plectin underlying EBS, collagen 7 underlying DEB, and laminins and plectin underlying JEB. Both recessive and dominant autosomal inheritance patterns exist, with recessive forms usually showing more severe phenotypes. In addition to epithelial defects, some forms are associated with other characteristics such as muscular dystrophy or pyloric atresia.



Research continues to reveal not only new mutations but also the importance of genotype-phenotype relationships in determining the characteristics and severity of any particular form.

## Caring or curing?

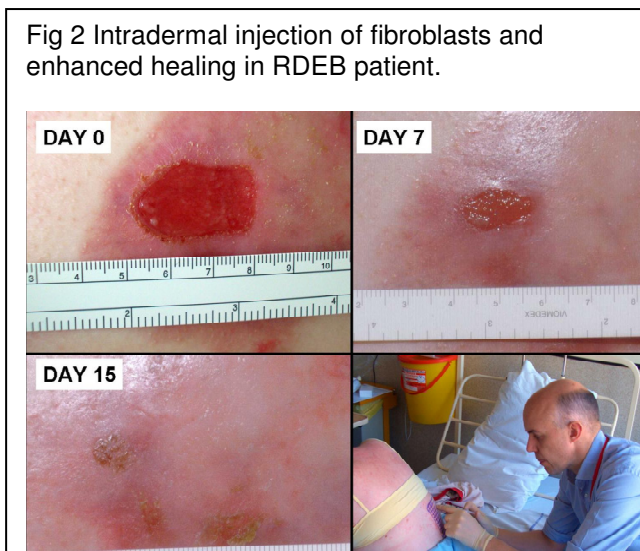
There is currently no cure for any form of EB, and treatment focuses largely on wound care by protective bandaging to reduce pain and further damage, and complications from the risks of infection, and fluid loss. The chronic wounds of EB can result in decreased mobility owing to pain and the extensive scar tissue that forms. Scarring in turn can result in constriction of the mouth or throat, or 'mitten' deformities of the hands and feet: the benefits of surgery to release fingers, for example, are of limited duration as scar tissue starts to form again immediately. For some types of EB, the internal mucosa is also affected, and may impact upon the full length of the GI tract, respiratory and reproductive tracts, and corneas. Nutrition can be compromised, resulting in osteoporosis, and general failure to thrive: quite young children can depend on gastrostomies or require highly specialized diets. Recessive Dystrophic Epidermolysis Bullosa (RDEB) is also characterized by the very high incidence of aggressive squamous cell carcinoma in teenagers and young adults, against which surgery, radio- or chemotherapy are not effective.

## What is fibroblast cell therapy?

Fibroblast cell therapy has, so far, been used in a pilot trial to target RDEB, where the defect in the Collagen 7 gene (diverse mutations result in defective or absent Collagen VII) results in the loss of the Collagen VII anchoring fibrils which are essential in maintaining adhesion between the epidermis and dermis\*.

Fibroblast cell therapy involves the intradermal injection of small volumes typically (0.25ml, containing up to 5 million cells per injection) of a suspension of cultured allogeneic neonate foreskin fibroblasts, at multiple sites around the edges of a wound (Fig 2).

A pilot trial of 13 patients with RDEB has proved remarkably beneficial: of the patients treated whose skin has been assessed in detail to date, all show improved healing of wounds, as well as increased skin strength, and reduced blistering.



A single injection of donor fibroblasts can lead to increased type VII collagen at the dermal-epidermal junction for several months without eliciting any adverse effects. Anecdotally, some patients in the pilot trial also reported reduction of scar tissue and increased mobility of affected joints. Fibroblast therapy is not a cure, but is the first treatment which results in an improvement in the condition of the EB patient and a major improvement in quality of life.

Fibroblasts from unrelated donors are not highly immunogenic, and are already used widely in grafts for treating burns, ulcers and following traumatic surgery: fibroblast therapy is not expected to have any major side-effects, and no side-effects have been reported in the pilot EB fibroblast therapy trial to date beyond mild transient reddening (very mild inflammation) at the site of injection for a few patients.

#### ***How does fibroblast therapy work?***

The mechanism(s) of action of the fibroblast therapy is(are) at present unknown, and DEBRA is funding further research into this, while at the same time promoting activities to progress to Phase II trials. The rationale for the pilot trial was that benefits of fibroblast injections had been observed in a large number of separate studies of RDEB mouse models, where injections of either mouse or human collagen VII, or healthy human or mouse fibroblasts resulting in de novo collagen synthesis from the injected fibroblasts, enhanced healing and reduced blistering, together with other studies by John McGrath's group (Ref 1). In RDEB patients, it is not known whether the benefits derive from de novo collagen synthesis from injected fibroblasts, upregulation of the patient's own faulty collagen VII, or a paracrine effect: the latter two possibilities are supported, respectively, by the observation that RDEB patients whose mutation still allows synthesis of some, albeit faulty, collagen benefited the most from the fibroblast injections; and that collagen VII synthesis was also upregulated in the layer of basal keratinocytes in the epidermis of these patients.

#### ***What alternatives to fibroblast therapy exist?***

Ideally, a therapy for EB would be a safe, once-only, systemically active, treatment that resulted in a lifetime cure. Such a therapy is still some way off, although emerging therapies for EB include gene-, protein-, cell- and drug-therapies and incremental advances are being made in each field.

Some therapeutic approaches target local healing (grafting or local cell injections), whereas others target systemic healing (bone-marrow transplants); some aim for temporary improvement (fibroblast or protein therapy), whereas others aim for permanent cure (bone-marrow transplant, or grafting of limited areas of skin). Most are a compromise between these factors, as well as safety and efficacy. For example, *ex vivo* gene therapy has so far only been tested in one patient for one form of EB (JEB) and while it has proved successful, with the skin of the grafted area strong, stable with no sign of rejection after three years (Ref 2), only the grafted area is 'cured', and several obstacles, both technical and regulatory, persist. DEBRA continues to fund research in gene therapy to address these issues.

Each therapeutic approach has advantages and disadvantages, and it is possible that different types of treatment, balancing benefit and risk for the important features of each type of EB, will be required. It is likely that dominant and recessive forms of EB will require different therapeutic approaches, and treatments not targeted to the primary pathology but to the downstream consequences such as poor wound healing or scarring, may also play a role.

Various forms of cell therapy are entering clinical trials: these include skin grafting in cases of revertant mosaicism, intradermal injections of allogeneic fibroblasts, intradermal or intravenous injections of allogeneic bone marrow mesenchymal stem cells, and allogeneic bone marrow transplants with or without umbilical cord cells or mesenchymal stem cells (Table1). Gene-, protein- and drug-therapies await clinical assessment but trials are pending.

### DEBRA's view

It is in this arena that DEBRA has considered that fibroblast therapy seems to represent, based on results to date, an expectedly safe treatment which offers patients the first opportunity for improved skin healing and reduced blistering with concomitant improvements in quality of life. At the same time DEBRA recognizes that fibroblast therapy as currently performed is probably a step in the direction of refined therapies, both local and systemic, derived from this and other therapeutic approaches. DEBRA therefore supports further development of fibroblast therapy through Phase II trials, and basic research to elucidate the mechanisms of fibroblast action, as well as fundamental, and preclinical research into diverse other potential therapeutic approaches.

Table 1		EB therapy technologies in development	Research stage/Clinical Trial
<b>Gene therapy</b>			
•	JEB Grafting ex-vivo Genetically modified		2006 pilot (1 patient)
•	RDEB Grafting ex-vivo Genetically modified (France)		?2010
•	RDEB Grafting ex-vivo Genetically modified (USA)		?2009/2010
<b>siRNA/ Gene Therapy</b>			
•	EBS		in development
<b>Cellular therapy - localized</b>			
•	RDEB Localized (fibroblast injections into skin) (UK)		Phase II ?2009/2010
•	RDEB Chimeric skin equivalents (fibroblasts from healthy donors + patient keratinocytes) (Spain)		Phase IIb ongoing
<b>Cellular therapy - systemic</b>			
•	RDEB Systemic bone-marrow (USA- Minnesota)		Phase I/II ongoing
•	RDEB Systemic bone-marrow (USA – New York)		Phase I/II started last week?
<b>Protein Therapy</b>			
•	RDEB Intradermal collagen (USA – California)		Phase I ?2009/2010
<b>Cell/Gene Therapy</b>			
•	JEB Grafting (autologous revertant) (Netherlands)		in development

\*Collagen 7 is a large gene, with, at 118, the greatest number of exons reported for any gene. Collagen VII is a homotrimer (ie composed of three identical polypeptide chains). Each chain has a central 145kDa triple-helical collagenous domain, and is flanked at the N-terminus by a large 145kDa non-collagenous domain (NC1), and at the carboxy terminus by a short 34kDa non-collagenous domain (NC2). Collagen VII forms dimers stabilized by sulphide bonds between the NC2 domains; NC2 domains are enzymatically cleaved off, and the dimers aggregate to form anchoring fibrils which then interact with various other fibrils to anchor the epidermis and basement membrane to the dermis. The NC1 domain interacts with fibronectin, collagen 1, laminin 5 and other extracellular matrix (ECM) proteins. There is currently debate over the possible role of NC1 in either promoting or preventing squamous cell carcinoma initiation, invasion or metastasis.

### References

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