Safety and feasibility of intrastromal injection of cultivated human corneal stromal keratocytes as cell-based therapy for corneal opacities
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Supplementary Materials

Supplementary Fig. S1. Intratromal cell injection. A. Rat cornea prior to injection. B. Creation of a stromal tunnel using a 31-gauge needle with a bevel-down approach. C. A stromal tunnel of ~0.5 mm stretch. D. Insertion of a blunt needle (31-gauge) connected to a Hamilton syringe. E. Slow injection of 2 μl cell suspension and bleb formation. F. Apply cotton swap to slightly pressurize the injection site for 3~5 sec to reduce backflow. G. Bleb formation after injection. H. Topical tobramycin after injection.
Supplementary Fig. S2. Effect of needle size on cell viability. Human SFs suspended in PBS at $10^4$/ml were delivered through needles of size (27, 30, 31 and 33-G) at a rate of 2 ml per sec onto collagen I-coated coverslips to reproduce the cell injection in vivo. After 6 hours for cell attachment, Calcein-AM cell viability assay was carried out. The cells passed through 27, 30 and 31-G had viabilities (>96%) indifferent to cells delivered using a P10 pipet. Significant reduction of cell viability was noted for cells passed through 33-G needle (24±11%). *P<0.05, Mann Whitney U test.
Supplementary Fig. S3. Ultrastructural observation of stromal integration of injected cells. A. Low magnification showing the rat stromal resident cells with close interaction between cells and stromal matrix. B. The regular collagen fibril pattern adjacent to the injected cells. C. The cytoplasmic inclusions containing Molday ION-Fe conjugates indicate the injected human cells. (Inset) cytoplasmic vesicle containing Fe conjugates under higher magnification. D. A close interaction of collagen fibrils or possible deposition of collagen fibrils by the injected cell.
Supplementary Fig. S4. Effect of cell injection on rat stromal resident cell viability.

Rat corneal sections at different weeks post-injection were subjected to TUNEL analysis using TMR-In Situ Cell Death Detection kit. Apoptosis signal (red fluorescence) was detected for the injected ION-labeled human cells (green fluorescence) and non-labeled rat stromal cells (without green fluorescence). All cell nuclei were stained with DAPI (blue fluorescence). A. Representative pictures at week 2 and 4 after cell injection and week 2 after PBS injection are shown, with higher magnification of injection site in central corneal region (white rectangle) displayed in right column. B. Quantification of TUNEL positive cells in ION-positive human cells and non-labeled rat stromal cells was performed and percentages of TUNEL cells in either population were calculated and plotted in bar chart. In all examination time points, the apoptosis rates of human cells (in average >10%) were significantly higher than that of rat stromal cells (<3%) on the same sections. The apoptosis rates of rat stromal cells were also low in PBS-injected corneas and normal rat corneas (<3%). *P<0.05 comparing the apoptotic index between the injected human cells and the rat stromal resident cells (Mann-Whitney U test).
Supplementary Fig. S5. Rat stromal changes at post injection. Immunofluorescence of keratan sulfate (red fluorescence) revealed the ground substance of corneal stroma. 

A and B. Corneas after cell injection showed distorted KS lamellae in the first 2 weeks with stromal gaps near to the injected human cells (ION labeled, green fluorescence). 

C. The stromal KS lamellar pattern was virtually restored after 5 weeks and the injected cells were closely interacted with stromal lamellae with regular KS pattern. 

D. Normal rat cornea displayed a regular KS lamellar pattern.