

Supplementary Online Content

Martinod E, Chouahnia K, Radu DM, et al. Feasibility of bioengineered tracheal and bronchial reconstruction using stented aortic matrices. *JAMA*.
doi:10.1001/jama.2018.4653

eMethods. Follow-up and outcome assessment

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Follow-up and outcome assessment

Cell proliferation from cryopreserved aortic allografts after thawing

In order to detect viable cells in cryopreserved aortic allografts, culture explants were performed from graft biopsies before implantation. Cell proliferation was analyzed by optical microscopy after adding fluorescein diacetate.

DNA extraction and PCR analysis

In order to detect the presence of donor cells (XY cells) from cryopreserved aortic allograft in the biopsy specimens from patient 2 fifteen months after implantation, genomic DNA for PCR analysis was prepared as previously described (Bensidhoum blood 2004). To detect the presence of human SRY gene, amplification of the DNA using TaqMan® gene expression assays encoding for human SRY gene (Hs00976796_s1; Applied Biosystem) and TaqMan® Universal PCR Master Mix (Applied Biosystems Inc) were performed using an iCycler thermocycling apparatus (MyiQ™ Single-Color Real-Time PCR; Bio-Rad Laboratories). The positive control for SRY DNA was isolated from human XY MSCs and the negative control for SRY DNA was isolated from human XX MSCs

Immunohistochemistry of type 2 collagen and Sox9

Biopsy specimens from patient 4 were fixed in 4% paraformaldehyde (pH = 7.4) for 36 hours and then embedded in paraffin and cut into 5µm sections, then stained with Hematoxylin-Eosin-Safran (HES) for histological examination. Immunodetection of type 2 collagen and Sox9 were performed using respectively goat polyclonal anti-collagen type 2 (Santa Cruz) and rabbit polyclonal anti-Sox9 (Sigma).