

Xeno-free defined conditions for culture of human embryonic stem cells, neural stem cells and dopaminergic neurons derived from them.

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Public Summary:

Scientific Abstract:

BACKGROUND: Human embryonic stem cells (hESCs) may provide an invaluable resource for regenerative medicine. To move hESCs towards the clinic it is important that cells with therapeutic potential be reproducibly generated under completely defined conditions. **METHODOLOGY/PRINCIPAL FINDINGS:** Here we report a four-step scalable process that is readily transferable to a Good Manufacture Practice (GMP) facility for the production of functional dopaminergic neurons from hESCs for potential clinical uses. We show that each of the steps (propagation of ESC-->generation of neural stem cells (NSC)-->induction of dopaminergic precursors-->maturation of dopaminergic neurons) could utilize xeno-free defined media and substrate, and that cells could be stored at intermediate stages in the process without losing their functional ability. Neurons generated by this process expressed midbrain and A9 dopaminergic markers and could be transplanted at an appropriate time point in development to survive after transplant. **CONCLUSIONS/SIGNIFICANCE:** hESCs and NSCs can be maintained in xeno-free defined media for a prolonged period of time while retaining their ability to differentiate into authentic dopaminergic neurons. Our defined medium system provides a path to a scalable GMP-applicable process of generation of dopaminergic neurons from hESCs for therapeutic applications, and a ready source of large numbers of neurons for potential screening applications.

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