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Ideal Xeno-Free Culture Conditions For Human Fibroblasts That Facilitate Efficient Reprogramming

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ABSTRACT

Patient derived human fibroblasts are the most widely used source for somatic reprogramming. Traditional methods require the use of fetal bovine serum containing medium for the isolation and expansion of these cells. As resulting induced pluripotent stem cells find their way towards clinical applications, xeno-free workflow requirements have become essential.

This study aims to identify ideal xeno-free media that best support fibroblast culture, health, and efficient gene transfer resulting in robust reprogramming. Human adult fibroblasts were cultured in five different xeno-free media systems, and their growth kinetics and cell health were measured in comparison to cells cultured in traditional FBS containing medium. Whole well imaging and continuous monitoring was carried out using IncuCyte® Zoom to track growth based on confluence. In addition, cells were transduced with a control GFP Sendai virus to assess the survival and percent GFP positive cells post transduction using the Attune™ Accoustic Cytometer. Culture conditions that best supported robust fibroblast growth and efficient Sendai transduction were further used for the generation of iPSC from different adult and neonatal human fibroblasts in Essential 8™ medium defined and feeder-free media. The identified xeno-free workflow offers flexibility and choices for patient sample processing to iPSC generation, critical for clinical-grade cells.

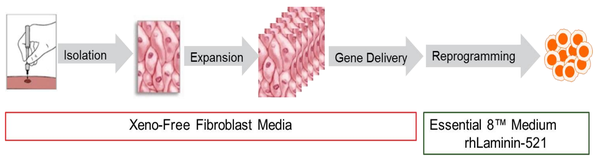
MATERIALS AND METHODS

•Growth kinetics was performed in order to study the health and growth of the fibroblast cells in different xeno-free media conditions. The cells were thawed in the control media containing FBS, and after the first passage, the cells were seeded into the 5 different xeno-free media conditions, as well as the control FBS media, and were cultured through passage four. At every passage, 100,000 cells were seeded per medium condition and a cell count was performed using a hemacytometer when the cells were harvested in order to determine the number of population doublings per passage.

•To study the cell's ability to be transduced by a virus, the CytoTune™ emGFP Transduction protocol was followed for the cells in the control and xeno-free media conditions.

•Reprogramming of fibroblast cells was carried out using the CytoTune™ iPS Sendai 2.0 kit according to manufacturers protocol. CytoTune™ 2.0 is a non-integrating reprogramming method which uses a modified version of the Sendai virus to deliver the Yamanaka factors into the cells. The virus replicates in the cytoplasm of the cell instead of the host nucleus. One modification to the protocol was made by re-plating the cells on day 4 instead of day 7.

•Episomal reprogramming of the fibroblast cells was carried out using the Epi5™ Episomal iPSC Reprogramming Kit and the NEON™ Transfection System together. The Epi5™ Episomal iPSC Reprogramming Kit uses a set of episomal vectors that carry the Yamanaka factors. The episomal reprogramming method is transgene-free and virus free.

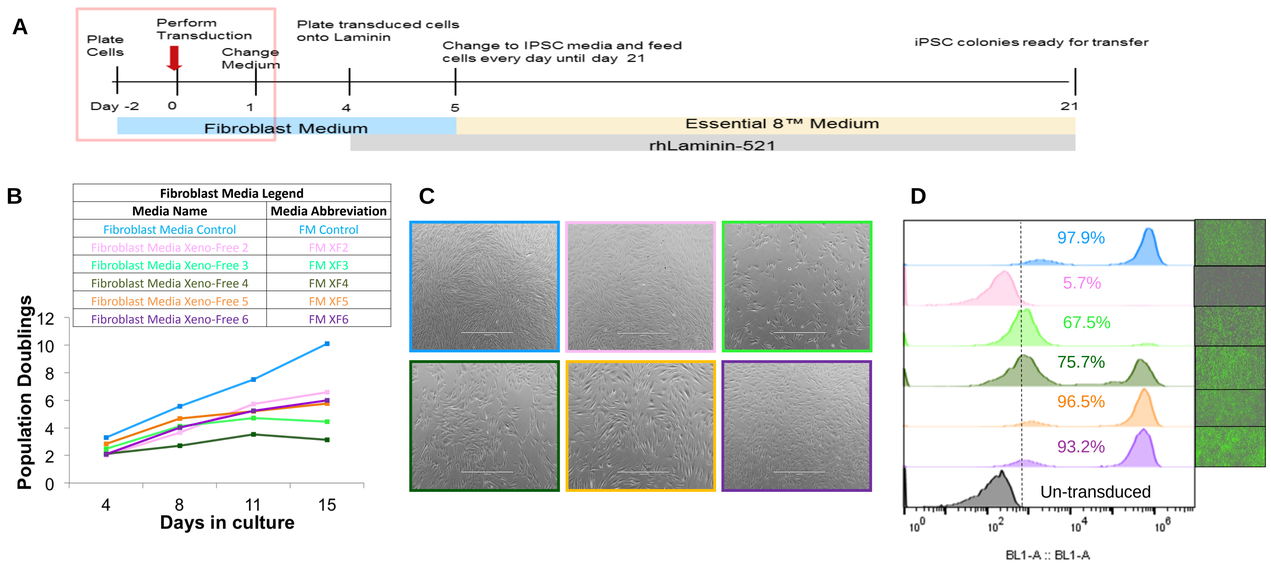


Fibroblast Study Metrics

Metric 1: Study xeno-free fibroblast survival and growth.
Metric 2: Study CytoTune™ emGFP transduction efficiency.
Metric 3: Study non-integrating, xeno-free reprogramming efficiencies.

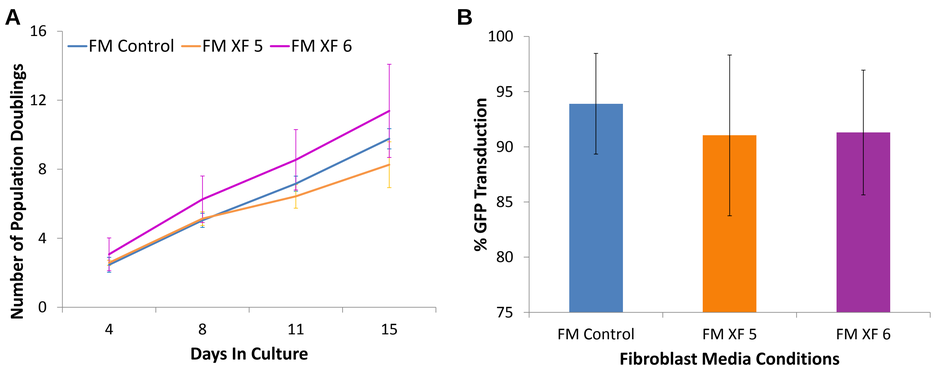
RESULTS

Figure 1. Fibroblast Growth Kinetics & CytoTune™ emGFP Transduction Efficiency Results in Xeno-Free Media



A. Workflow schematic of fibroblast reprogramming with CytoTune™. Highlighted box focuses on fibroblast growth/manipulation with xeno-free media
B. Population doublings over 4 passages for the fibroblast cells in xeno-free media conditions.
C. Images, taken with the EVOS™ FL Cell Imaging System, of fibroblast cells cultured in the control FBS media and xeno-free media conditions.
D. CytoTune™ emGFP transduction efficiency results on xeno-free media conditions.

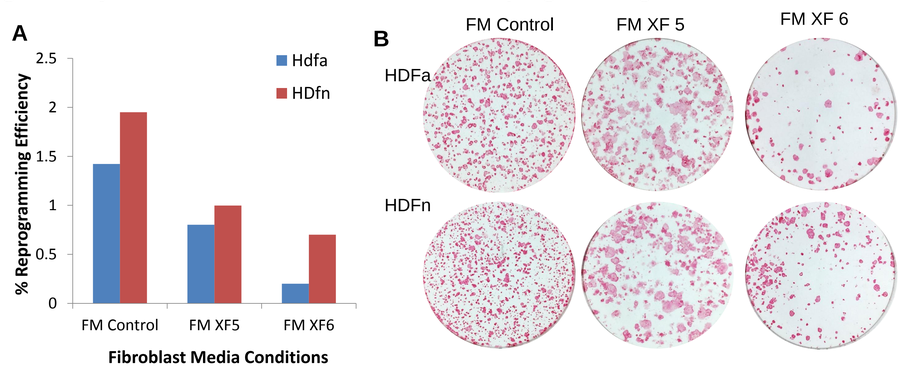
Figure 2. Xeno-Free Fibroblast Growth Kinetics & CytoTune™ emGFP Transduction Efficiency Across Donors



A. The average population doublings for 3 different HDFa lots over 4 passages of the fibroblast cells in xeno-free media conditions 5 and 6.

A. The average CytoTune™ emGFP transduction efficiency results on 3 different HDFa lot numbers, tested in xeno-free media conditions 5 and 6.

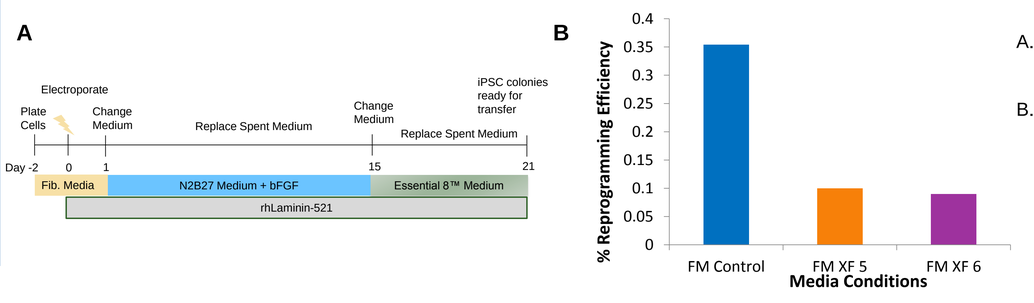
Figure 3. CytoTune™ 2.0 Xeno-free Fibroblast Reprogramming Results



A. Reprogramming efficiency of adult and neonatal human dermal fibroblast cells in xeno-free fibroblast media conditions 5 and 6 using CytoTune™ 2.0 reprogramming method.

B. Images of alkaline phosphatase positive colonies derived from CytoTune™ 2.0 reprogramming from adult (HDFa) and neonatal (HDFn) fibroblast cells in traditional Essential 8™ medium.

Figure 4. Effect of Xeno-Free Media Conditions on Fibroblast Reprogramming with Episomal Vectors



A. Schematics of Episomal reprogramming workflow

B. Episomal reprogramming efficiency of fibroblasts in xeno-free media conditions 5, and 6 using the Epi5™ reprogramming kit.

CONCLUSIONS

With induced pluripotent stem cells making their way toward clinical applications there is a need to develop and stream line a xeno-free work flow for the development of IPSC's. Developing a xeno-free workflow has its challenges hence we have set forth to determine ideal xeno-free reprogramming conditions for human dermal fibroblast (HDF) cells.

Of the five xeno-free media formulations tested in fibroblast growth kinetics xeno-free medias 2, 5, and 6 showed that they supported fibroblast growth and health but less efficiency than the control FBS medium. During the CytoTune™ emGFP transduction, it was determined that xeno-free medias 5 and media 6 were able to support fibroblast cell transduction relatively close to the control FBS media. Therefore, xeno-free medias 5 and 6 were chosen to move forward and be studied across different fibroblast lot numbers in order to determine whether they can support fibroblast growth kinetics and transduction across different donors.

Growth kinetics and CytoTune™ emGFP Transduction efficiency were then studied in two different HDFa lot numbers. Both growth kinetics results as well as transduction efficiency results showed that xeno-free media conditions 5 and 6 support fibroblast growth and transduction across different HDFa lot numbers and these two media conditions were chosen to be studied in fibroblast reprogramming.

CytoTune™ 2.0 is a non-integrating reprogramming method which was chosen to study xeno-free fibroblast reprogramming. It was noticed that while xeno-free media conditions 5 and 6 allowed reprogramming of fibroblast cells into iPSCs, the reprogramming efficiency showed a reduction when compared to the control FBS medium. Medium 5 also provided a higher reprogramming efficiency than medium 6.

For the episomal reprogramming, xeno-free media conditions 5 and 6 had a similar reprogramming efficiency; however, it was significantly less than the control FBS media.

For current studies and future directions; xeno-free media conditions 5 and 6 will be tested in fibroblast reprogramming using different HDFa lot numbers and small molecules will be tested to try to enhance reprogramming efficiencies. For reprogramming using episomal vectors, xeno-free medias 5 and 6 will be tested with Essential 8™ medium similar to the CytoTune™ 2.0 workflow.

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