Use of non-modified RNAs for the derivation of clinically-relevant iPS cell lines from human blood, urine and skin cells using GMP-compliant reagents

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Introduction

In 2015 we published the unique application of a non-modified RNA technology to the reprogramming of human adult fibroblasts and human blood-derived endothelial progenitor cells (EPCs)[1]. Human blood provides easy access to adult human cell types for reprogramming purposes. Notably, EPCs can be clonally isolated from fresh or frozen mononuclear cell (MNC) preparations from only 10 mL of either human peripheral or cord blood (Figure 1B). The adherent nature and high proliferative capacity of EPCs makes them highly desirable for repeated transfection with RNA when compared to commonly isolated hematopoietic suspension cell types. Furthermore, urine sampling provides perhaps the most non-invasive form of cell procurement. Urine-derived epithelial cells (UDCs) can be highly reproducibly isolated from only 30 mL of urine (Figure 1C) . Here we present a flexible, yet powerful, RNA-based reprogramming method that combines a novel cocktail of synthetic, non-modified reprogramming [OCT4, SOX2, KLF4, cMYC, NANOG and LIN28 (OSKMNL)] and immune evasion mRNAs [E3, K3, B18] with reprogramming-enhancing mature, double-stranded microRNAs from the 302/367 cluster. Inclusion of the E3, K3, and B18 immune evasion mRNAs in the RNA transfection cocktail eliminates the need to supplement cell culture medium with recombinant B18 protein during the reprogramming process. This unique combination of different RNAs results in a highly efficient and robust reprogramming protocol using only GMP-compliant substrates (iMatrix-511 and vitronectin), media compositions (xeno-free medium or human serum supplementation) and RNA to produce clinically-relevant iPS cells (Figure 2A-C). Elevated Oct4 transcript levels in the RNA cocktail resulted in transfection protocols that efficiently generated TRA-1-60 positive iPS cell colonies from human adult and neonatal fibroblasts (up to 4%), blood-derived EPCs (up to 0.04%), HUVECs (up to 3%) UDCs (up to 0.5%) within 10 days (Figure 3 A-C and Table 1). Additionally, these different iPS cell lines demonstrate highly consistent cardiomyocyte, neural and early endoderm differentiation potential (Figure 4 A-C). The unique combined application of non-modified RNAs, using GMP-compliant reagents, for the cellular reprogramming of different human cell lines results in clinically-relevant iPS cells that are well suited for consistent application of *in vitro* differentiation protocols.

HUMAN FIBROBLAST

HUMAN BLOOD-DERIVED ENDOTHELIAL CELLS (EPCs)

HUMAN URINE-DERIVED EPITHELIAL CELLS (UDCs)

Establishment & Immunocytochemical (ICC) Characterization

Figure 1A

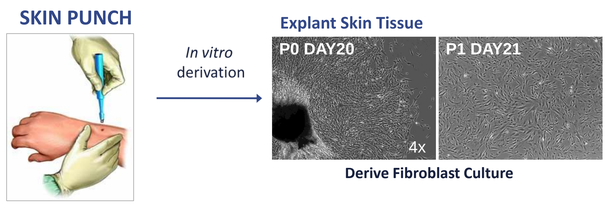

FIGURE 1A: Derivation of dermal fibroblast lines from a skin biopsy in 21 days.

B

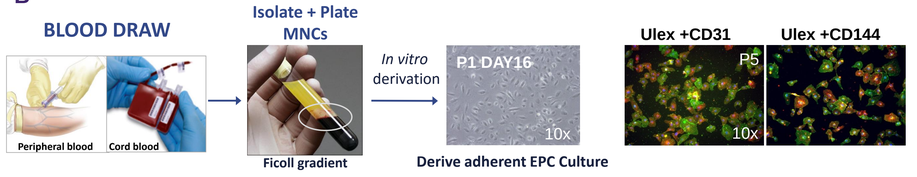

FIGURE 1B: EPCs derivation from peripheral blood or cord blood and ICC of EPCs. Human mononuclear cells (MNCs) isolated from a minimum of 10 mL blood were plated on human collagen and cultured in EPC Derivation Medium, containing 20% human serum. EPCs at P5 were stained with the appropriate antibodies and DAPI for visualization. Merged images are shown.

C

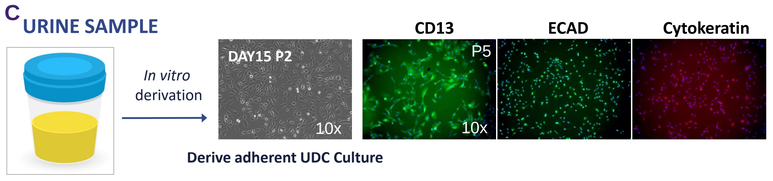

FIGURE 1C: UDCs derivation from urine and ICC. A minimum of 30 mL of washed, pelleted urine cells onto iMatrix-511 and cultured in UDC Derivation medium containing 10% human serum. UDCs at P5 were stained with the appropriate antibodies and DAPI for visualization. Merged images are shown.

Protocol Timeline & Morphology Progression for Reprogramming with StemRNA-NM Kit

Figure 2A

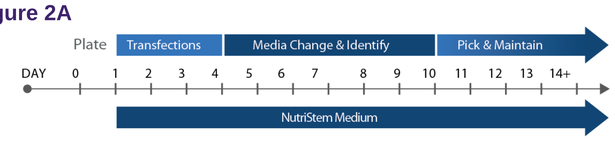

FIGURE 2A: Timeline for the reprogramming of human fibroblasts using the StemRNA-NM Kit. 1x10[5] human fibroblast were seeded onto iMatrix-511 in NutriStem™ XF/FF Culture Medium reprogrammed into iPS with only 4 total daily RNA transfections.

B

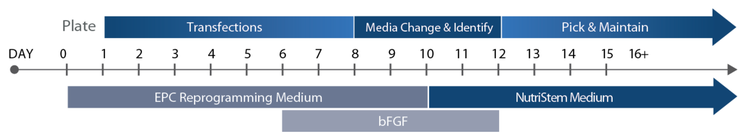

FIGURE 2B: Timeline for the reprogramming of human EPCs using the StemRNA-NM Kit. 1x10[5] EPCs were seeded onto iMatrix-511 in EPC-Reprogramming Medium containing 10% human serum. On day 10 culture medium was transitioned to NutriStem XF/FF Culture Medium .

C

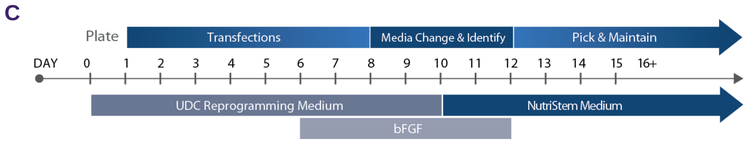

FIGURE 2C: Timeline for the reprogramming of human UDCs using the StemRNA-NM Kit. 5x10[4] UDCs were seeded onto iMatrix-511 in UDC-Reprogramming medium containing 10% human serum. On day 10 culture medium was transitioned to NutriStem XF/FF Culture Medium .

Figure 3A

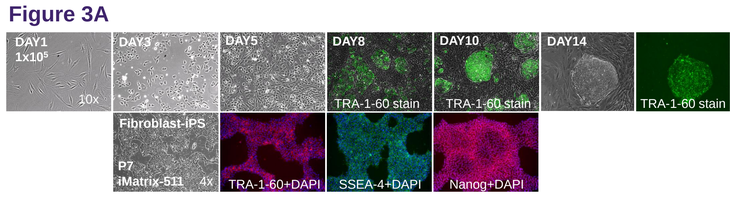

FIGURE 3A: Primary reprogramming culture morphology progression, resulting from the reprogramming of adult fibroblasts with the StemRNA-NM Kit on iMatrix-511 in NutriStem XF/FF. Day 8,10 and Day 14 primary Fibroblast-RNA-iPS cell colonies were identified using Stemgent StainAlive™ TRA-1-60 antibody and are able to be isolated from the primary culture between Day 10-14. Fibroblast-RNA-iPS cells were expanded on iMatrix-511 in NutriStem XF/FF and stained for pluripotency associated genes at P7 by ICC.

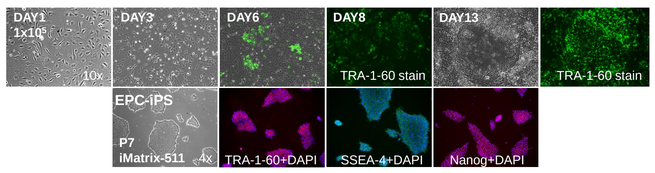

FIGURE 3B: Primary reprogramming culture morphology progression, resulting from the reprogramming of EPCs with StemRNA-NM Kit on iMatrix-511 and EPC-Reprogramming medium containing human serum. Day 6, 8, 13 primary EPC-RNA-iPS cell colonies were identified using Stemgent StainAlive TRA-1-60 antibody and are able to be isolated from the primary culture by Day 12-14. EPC-RNA-iPS cells were expanded on iMatrix-511 in NutriStem XF/FF and stained for pluripotency associated genes at P6 by ICC.

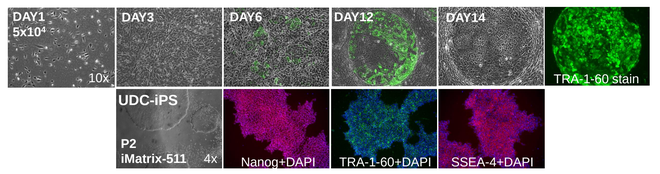

FIGURE 3C: Primary reprogramming culture morphology progression, resulting from the reprogramming of UDCs with StemRNA-NM Kit on iMatrix-511 and UDC-Reprogramming medium containing human serum. Day 6, 12, 14 primary UDC-RNA-iPS cell colonies were identified using Stemgent StainAlive TRA-1-60 antibody and are able to be isolated from the primary culture by Day 12-14. UDC-RNA-iPS cells were expanded on iMatrix-511 in NutriStem XF/FF and stained for pluripotency associated genes at P7 by ICC.

Characterization of RNA-iPS cells

Figure 4A

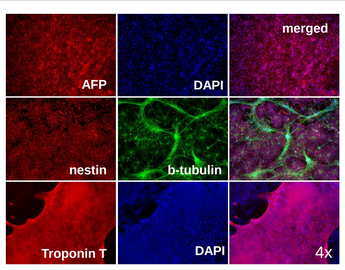

FIGURE 4A: *In vitro* differentiation of P8 Fibroblast-RNA-iPS cells expanded on iMatrix-511 were differentiated into early endoderm (AFP in red), neuronal cells (nestin in red; b-tubulin in green) and cardiomyocytes (Troponin T in red), DAPI (blue).

B

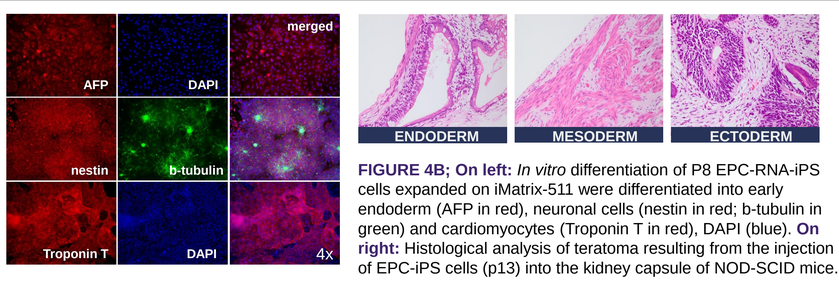

FIGURE 4B; On left: *In vitro* differentiation of P8 EPC-RNA-iPS cells expanded on iMatrix-511 were differentiated into early endoderm (AFP in red), neuronal cells (nestin in red; b-tubulin in green) and cardiomyocytes (Troponin T in red), DAPI (blue). **On right:** Histological analysis of teratoma resulting from the injection of EPC-iPS cells (p13) into the kidney capsule of NOD-SCID mice.

C

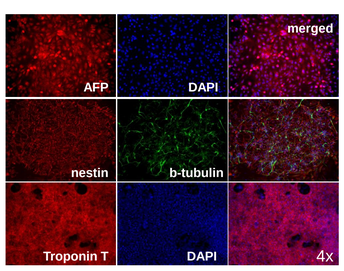

FIGURE 4C: *In vitro* differentiation of P11 UDC-RNA-iPS cells expanded on iMatrix-511 were differentiated into early endoderm (AFP in red), neuronal cells (nestin in red; b-tubulin in green) and cardiomyocytes (Troponin T in red), DAPI (blue).

StemRNA-NM Kit Features

Table1	NON-MODIFIED RNA REPROGRAMMING
Fibroblast	Yes
Blood cells (EPCs)	Yes
Urine-derived epithelial cells (UDCs)	Yes
# of transfections Fibroblasts (adult and neonatal)	4
# of transfections EPCs and UDCs	6-8
Days to primary iPS cell colonies	10-15 days
Reprogramming efficiency	2-4% (Fibroblasts) - up to 1000 colonies/6-well 0.4-3% (EPCs) - up to 200 colonies/6-well 0.1%- 0.5% (UDCs) - up to 20 colonies/6-well
Screening required	No

Summary

Generation of stable, pluripotent human iPS cell lines using StemRNA-NM Kit (#00-0076)

Reprogramming Protocols for:
- Human fibroblasts
- Blood-derived endothelial progenitor cells (EPCs)
- Urine-derived epithelial cells (UDCs)

Reprogramming Protocols do not require:
- Feeders
- Conditioned medium
- Small molecules
- B18 recombinant protein
- Splitting of ongoing reprogramming cultures

GMP-compatible Reprogramming Platform
- Xeno-free reprogramming reagents using human serum and iMatrix-511
- Non-modified RNAs synthesized under GMP-compliant protocol

References

1. Poleganov *et al* (2015) Efficient Reprogramming of Human Fibroblasts and Blood-Derived Endothelial Progenitor Cells Using Non modified RNA for Reprogramming and Immune Evasion. *Hum Gene Ther*. 11:751-66

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