Therapeutic Cloning: A New Vision of Somatic Cell Nuclear Transfer

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Introduction

- **Somatic Cell Nuclear Transfer** has been proposed as a tool to replace tissue or organ transplantation.
- This technique is based on the **introduction of a somatic cell nucleus intro an oocyte cytoplasm** (previously removed its nucleus) to reprogram it into a pluripotent state.
- It's possible thanks to the **oocyte cytoplasmic reprogramming factors.**

Methodology

- Data come from papers and reviews researched on PubMed database: **Scientific literature.**
- **Papers selection**: key word were introduced in order to search the most relevant papers. Papers were selected according to their historical importance, data of publication and journal.
- **Key words**: Somatic Cell Nuclear Transfer, Therapeutic Cloning, Human, Embryonic Stem Cell, Oocyte, Ethic.

Cloning over the years

Table 1. Human Somatic Cell Nuclear Transfer Over Years (*Drawn by author*)

Oocytes	Caryoplast	Enucleation	Transfer	Fusion	Activation	Achivement	Reference
Oocytes from a FIV center	ESC nucleus	Pipette compression	Perivitelline space injection	Electrofusion	I/DMAP	Degenerated blastocyst	*2005*
FIV patients donations	Adult fibroblasts	Extrusion or aspiration	Perivitelline space injection	Electrofusion	CI+: 1)HSA+6-DMAP 2)CHX+CYTD	Blactocyst development	*2008*
FIV patients donations	Fetus fibroblasts	Piezo-Assisted method	Cytoplasm injection	X	Electric and chemical activation	Blastocyst development from A and B group	*2009*
Oocyte donors	3-year-old boy fibroblasts	Oosight + Zilos	Perivitelline space injection	Electrofusion	Electrofusion	Five blastocyst	*2009*
Oocyte donors	Anonymously fibroblasts	Piezo-Assisted method	Cytoplasm injection	X	I/DMAP and TSA	I/DMAP and TSA	*2011*
Oocyte donors	Fibroblasts	Extrusion	Perivitelline space injection	Electrofusion	I/DMAP	Two-triploid hNT-ESC lines	*2011*
Oocyte donors	Fetal fibroblasts	Cytoplasm injection and extraction	HVJ-E into perivitelline space	X	30' after fusion: electropulse, 6-DMAP, TSA and caffeine	Stable hNT-ESC lines	*2013*
Oocyte donors	Fibroblasts from 35- and 75-year-old males	Cytoplasm injection and extraction	HVJ-E into perivitelline space	X	2h after fusion: electropulse, 6-DMAP, TSA and caffeine	Diploid hNT-ESC line	*2014*
Oocyte donors	Fibroblasts from a female with type 1 diabetes	Data not shown	Data not shown	Sendai virus with calcium-free medium	Electropulse, puromycin and 6-DMAP. FBS for hNT-ESC lines derivation	hNT-ESC line and differentiation into beta-pancreatic cells	*2014*

Therapeutic Cloning

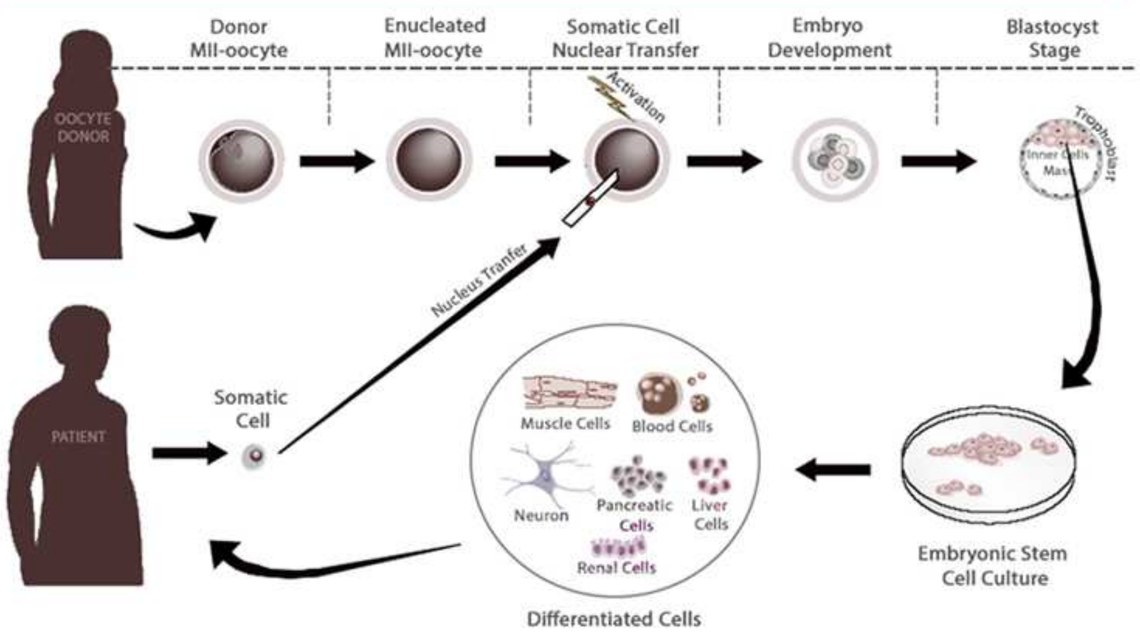

Image 1. Human therapeutic cloning. When a somatic cell nucleus is obtained from a patient, it could be transferred to an enucleated oocyte to be developed until blastocyst. Once reached, ICM may be isolated and cultured to achieve a hNT-ESC line which could be differentiated in the cell type needed by the patient. *(Drawn by author).*

Limitations

Practical considerations
- Efficacy of hSCNT remains low
- Impossibility of having large reserves of oocytes
- hNT-ESC maintain epigenetic memory

Ethical controversy
- Humanity status or dignity of the embryo
- *Slippery Slope* principe
- Oocyte's source and morality of paying for donations

Future alternatives

Interspecies Somatic Cell Nuclear Transfer

- Interspecies transfer of human fibroblast into enucleated bovine, mouse and rabbit oocytes.
- Comparisons between human and interspecies SCNT show aberrant pluripotency gene expression pattern

Nuclear Transfer to Mitotic Arrested Embryos

- Mice: Presence of cytoplasmatic reprogramming factors in both zygotes at interphase and metaphase as at two-cell embryos.
- Human: SCNT in embryos resulted to an arrest at morula stage because of a lack of reprogramming.

Conclusions

Human somatic cell nuclear transfer is a technique with a long history since 1952 that, recently, has allowed the derivation of human embryonic stem cells lines. Even though hNT-ESC and, therefore therapeutic cloning, are a really promising therapy; there's still a great deal of research to achive high-quality hNT-ESCs that would be suitable for tissue replacement therapy.