Effect of different Cryopreservation Media on Human Nucleus Pulposus Cells' Viability and Trilineage Potential

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Disclosures

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Introduction

- Over the past decade, progenitor cells capable of trilineage differentiation have been discovered within the nucleus pulposus (NP) of human intervertebral discs (IVDs).[1,2,3] These cells could have the potential to regenerate the IVD. Therefore, once isolated, a cost efficient method to store and preserve them has to be found.
- However, in the context of cell therapy, little is known about the effect of cryopreservation and expansion on here called „heterogenic" human NP cells (hNPCs).

Study aims

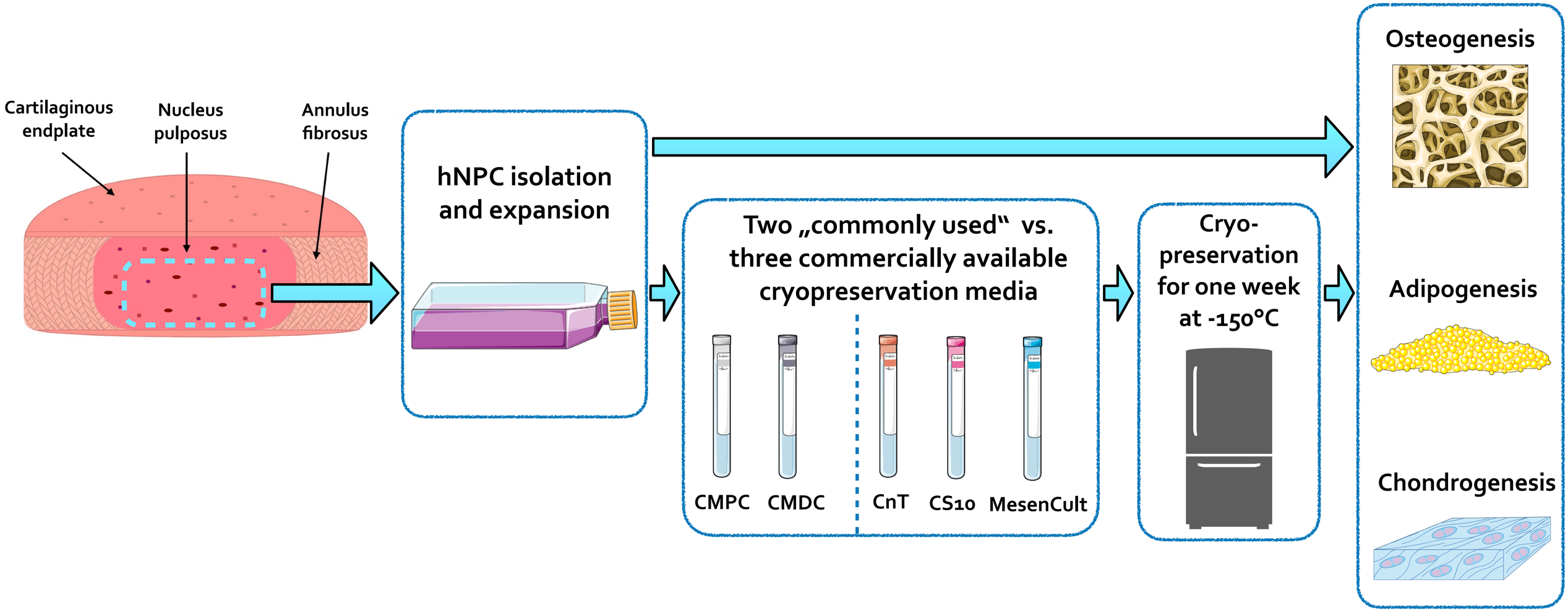
- Find an optimal approach to cryo-preserve hNPCs.
- Expand hNPCs and to investigate their trilineage differentiation potential before and after cryopreservation.

[1] Sakai D et al. (2012) *Nature Communications*

[2] Blanco J et al. (2010) SPINE

[3] Tekari A et al. (2016) *Stem Cell Research & Therapy*

Methods


Cartilaginous endplate
Nucleus pulposus
Annulus fibrosus
hNPC isolation and expansion
Two „commonly used" vs. three commercially available cryopreservation media
CMPC
CMDC
CnT
CS10
MesenCult
Cryo-preservation for one week at -150°C
Osteogenesis
Adipogenesis
Chondrogenesis

Results

Cell viability after cryopreservation

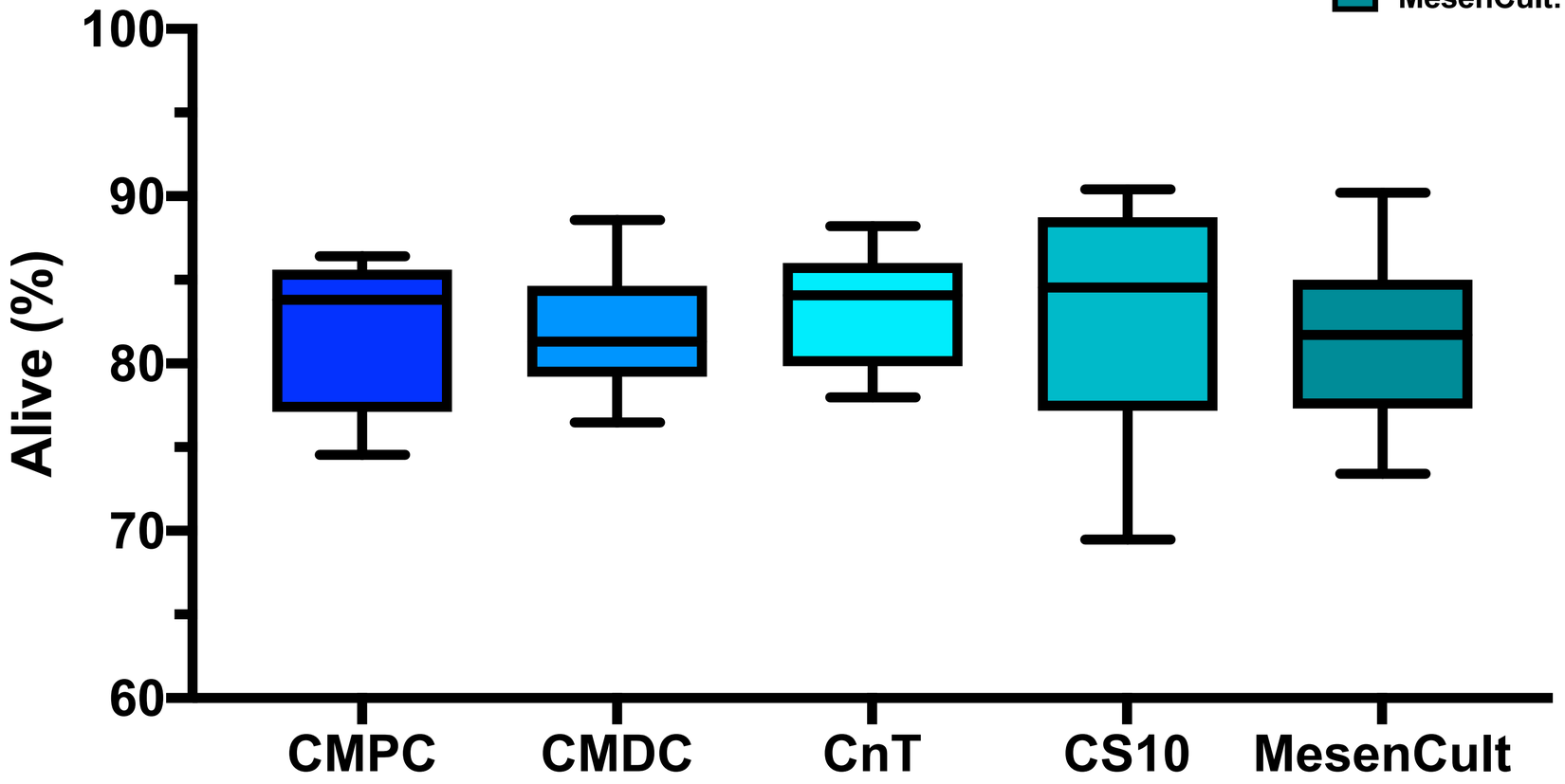
CMPC: Commonly applied cryo-medium for progenitor cells

CMDC: Commonly applied cryo-medium for differentiated cells

CnT: CellnTec Cryo-Defined Freezing Medium

CS10: cGMP-manufactured CryoStor® CS10

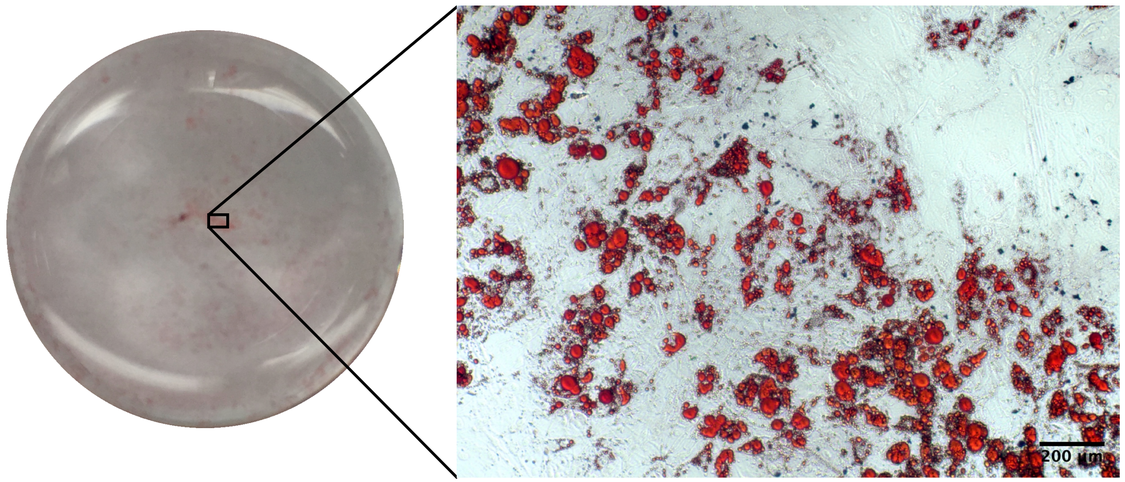
MesenCult: MesenCult™ -ACF Freezing Medium



The cell viability was the same for the cryopreservation media tested.

Results

Adipogenesis



Lipid droplets produced by hNPCs and stained with Oil Red O.

Fresh cells: Previously unfrozen cells

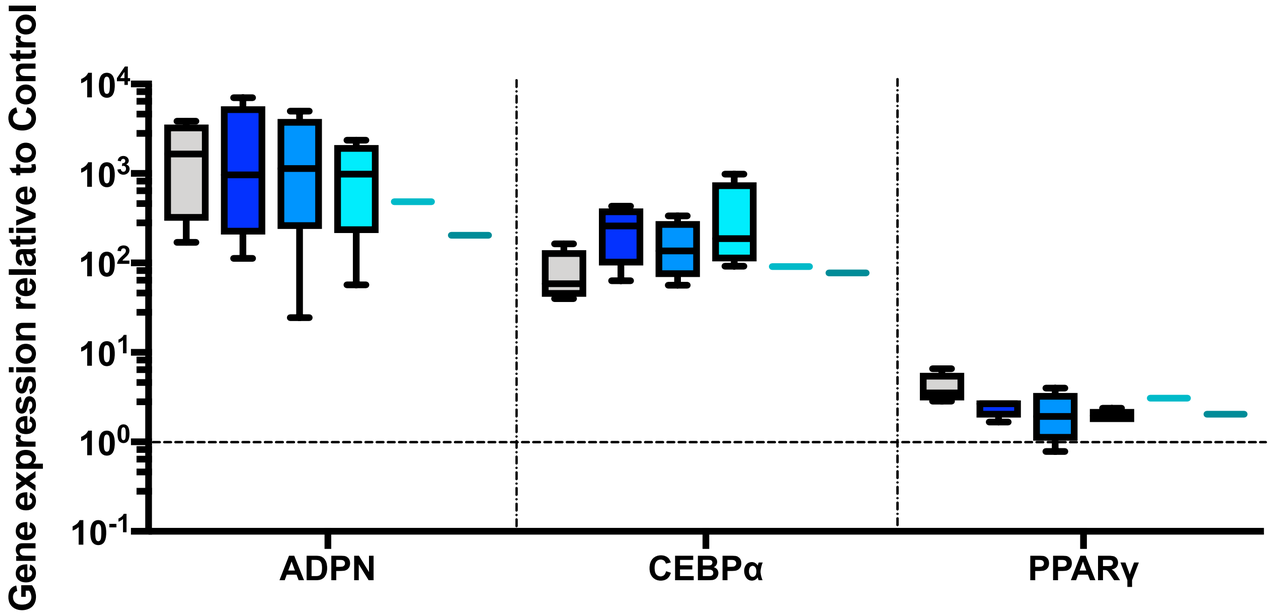
CMPC: Commonly applied cryo-medium for progenitor cells

CMDC: Commonly applied cryo-medium for differentiated cells

CnT: CellnTec Cryo-Defined Freezing Medium

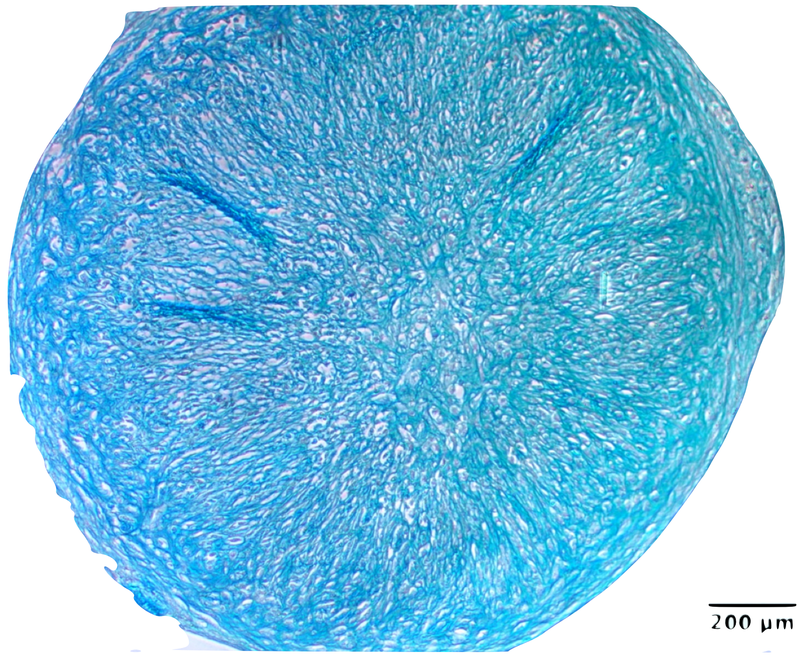
CS10: cGMP-manufactured CryoStor® CS10

MesenCult: MesenCult™ -ACF Freezing Medium



Results

Chondrogenesis



7 µm section of a hNPC pellet and stained with Alcian blue.

Fresh cells: Previously unfrozen cells

CMPC: Commonly applied cryo-medium for progenitor cells

CMDC: Commonly applied cryo-medium for differentiated cells

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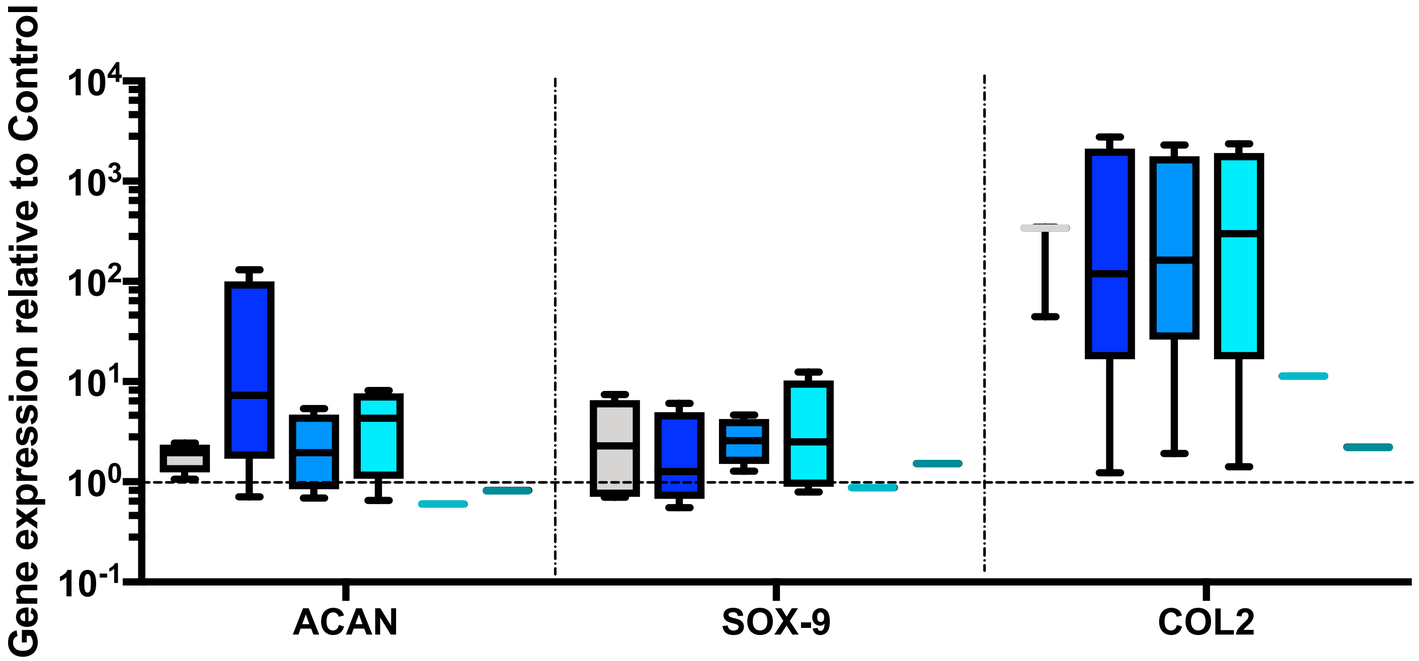


Results

Osteogenesis

Fresh cells: Previously unfrozen cells

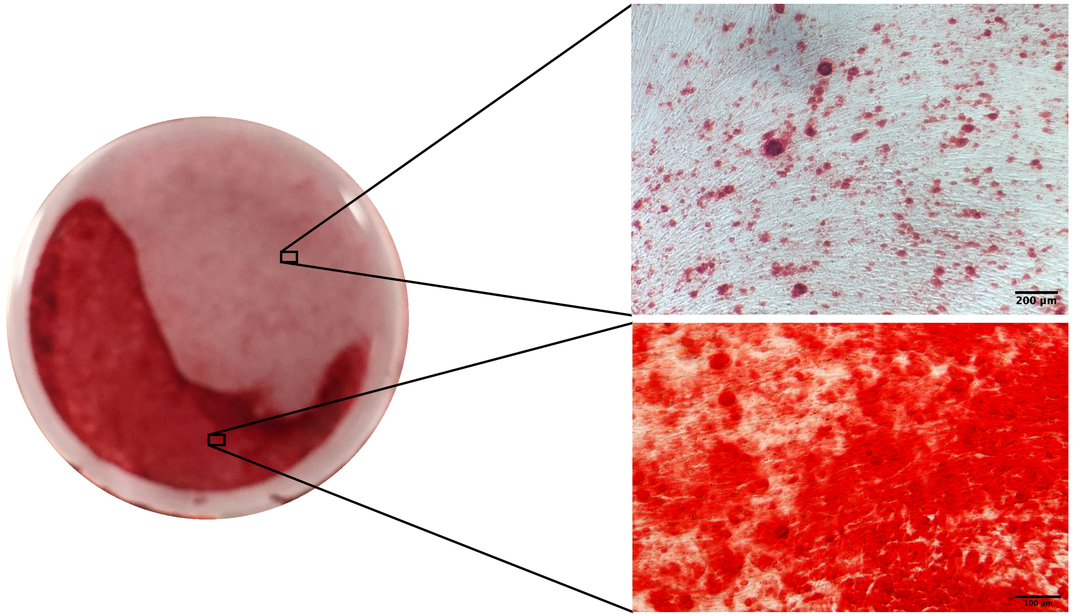
CMPC: Commonly applied cryo-medium for progenitor cells

CMDC: Commonly applied cryo-medium for differentiated cells

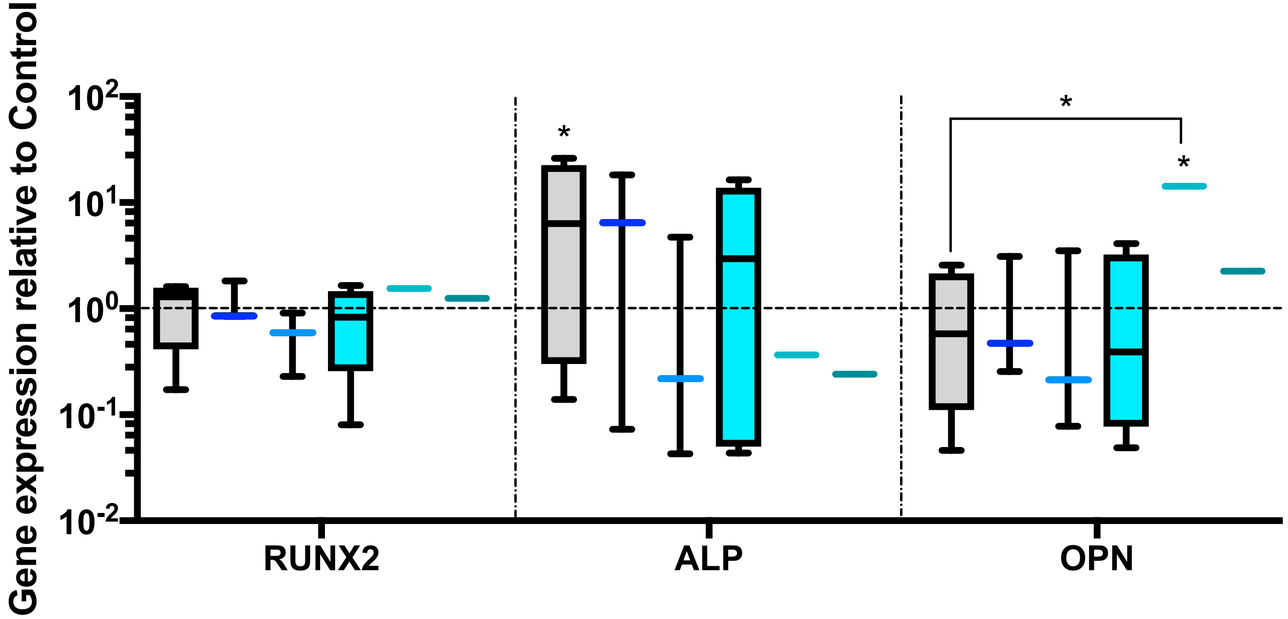
CnT: CellnTec Cryo-Defined Freezing Medium

CS10: cGMP-manufactured CryoStor® CS10

MesenCult: MesenCult™ -ACF Freezing Medium



Matrix mineralization of hNPCs and stained with Alizarin Red.



Discussion

- This study showed that hNPCs can be cryo-preserved with „commonly used“ cryopreservation media just as well as with commercially available media without any significant differences concerning cell viability. This is also interesting from an economical point of view, as „commonly used“ cryopreservation media are potentially more affordable than commercially available media.
- Moreover, hNPCs showed evidence of trilineage differentiation before and after cryopreservation. However, the trilineage potential was subjected to great donor variations. This inconsistency could be due to a varying yield of progenitor and/or stem cells in the NP tissue of each human donor.

Summary points

- „Commonly used“ cryopreservation media are just as efficient as commercially available media in terms of cell viability for hNPCs.
- hNPCs show evidence to undergo osteogenesis, adipogenesis and chondrogenesis to a certain extent, even after cryopreservation at -150°C for one week.