The effects of 3D culture on the expansion and maintenance of nucleus pulposus progenitor cell multipotency

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Disclosures

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Introduction

Low back pain (LBP) is a global health concern. Increasing evidence implicates **intervertebral disk** (IVD) **degeneration** as a major contributor. In this respect, **tissue-specific progenitors** may play a crucial role in tissue regeneration, as these cells are **perfectly adapted to their niche**. Recently, a novel progenitor cell population was described in the **nucleus pulposus** (NP) that is positive for **Tie2 marker**. These cells have **self-renewal capacity** and *in vitro* **multipotency** potential. However, extremely low numbers of the NP progenitors limit the feasibility of **cell therapy strategies**.

Study aims

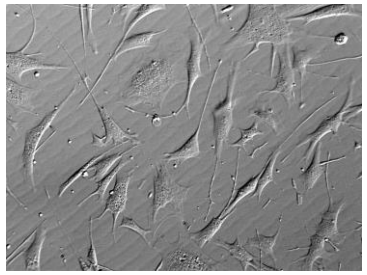
Here, we studied the influence of the **culture method** and of the microenvironment on the **proliferation rate** and the **differentiation potential** of **human NP progenitors** *in vitro*.

Methods

Phase 1 : NP digestion and cells harvesting

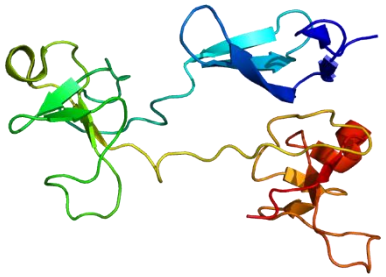
Dissociation and digestion of the Nucleus Pulposus (NP).

Phase 2 : Cells seeding in 2D or 3D



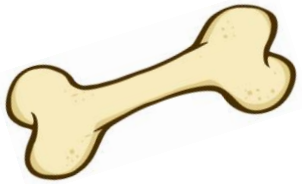
NP cells were seeded in 2D tissue culture flask or in 3D within alginate beads.

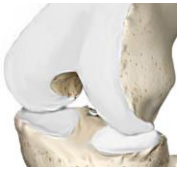
Phase 3 : Cells expansion on fibronectin coated plates

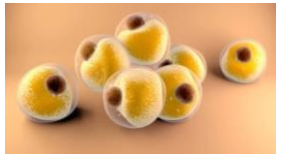


Cells were seeded onto tissue culture flasks coated with fibronectin

Phase 4 : Cells differentiation into tri-lineage



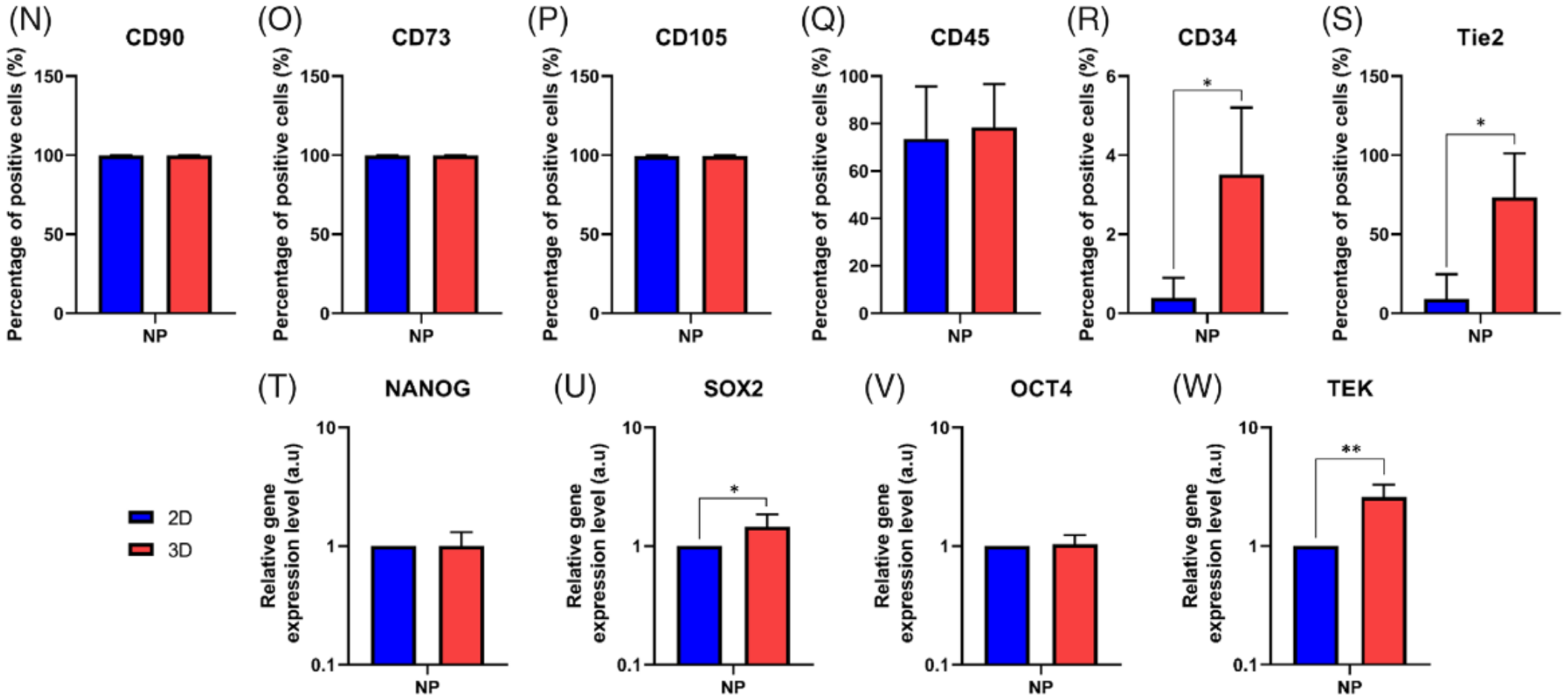




Moreover, experiments using **Tie2**$^{+}$ and **Tie2**$^{-}$ NP cells were also performed.

Results

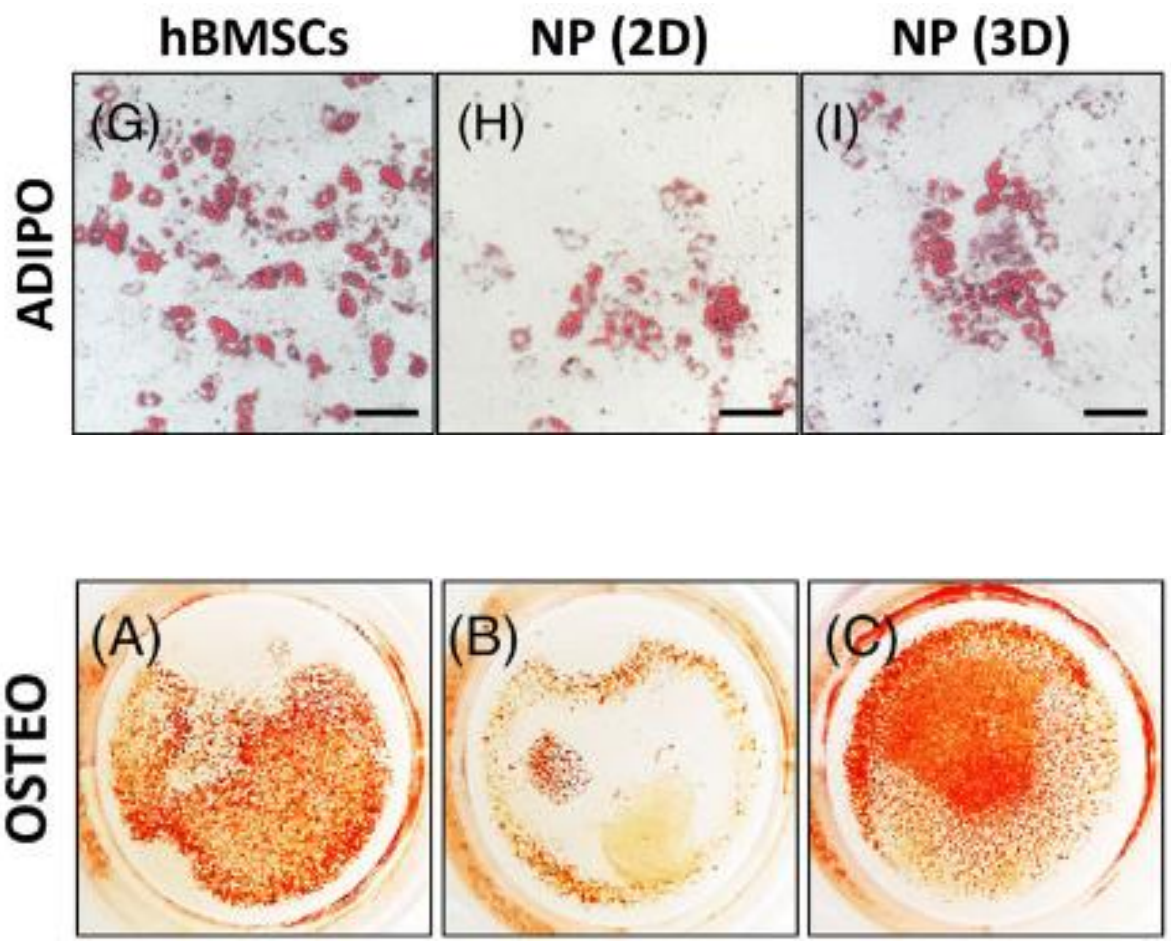
Flow cytometry and qPCR analysis

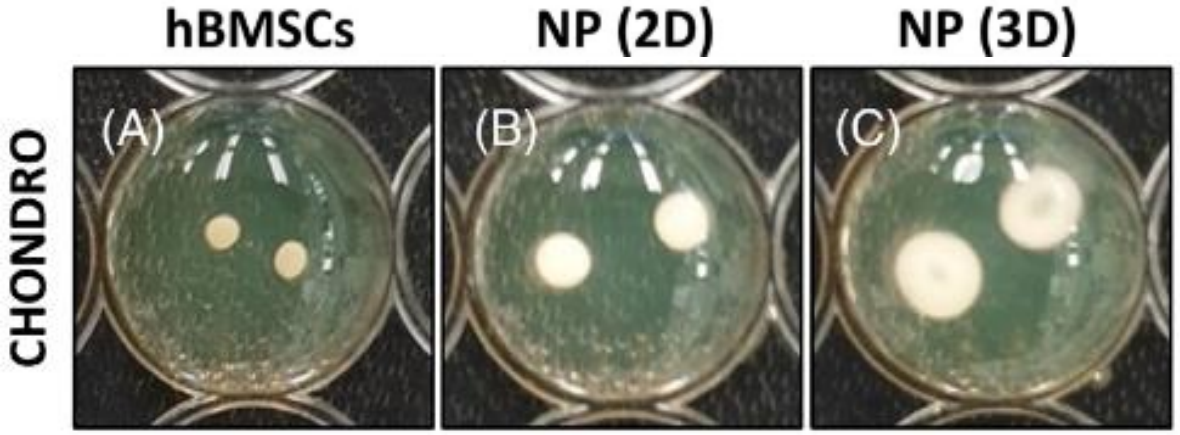


NP cells cultured in 2D vs 3D, at the end of Phase 3.

Results

Histological analysis

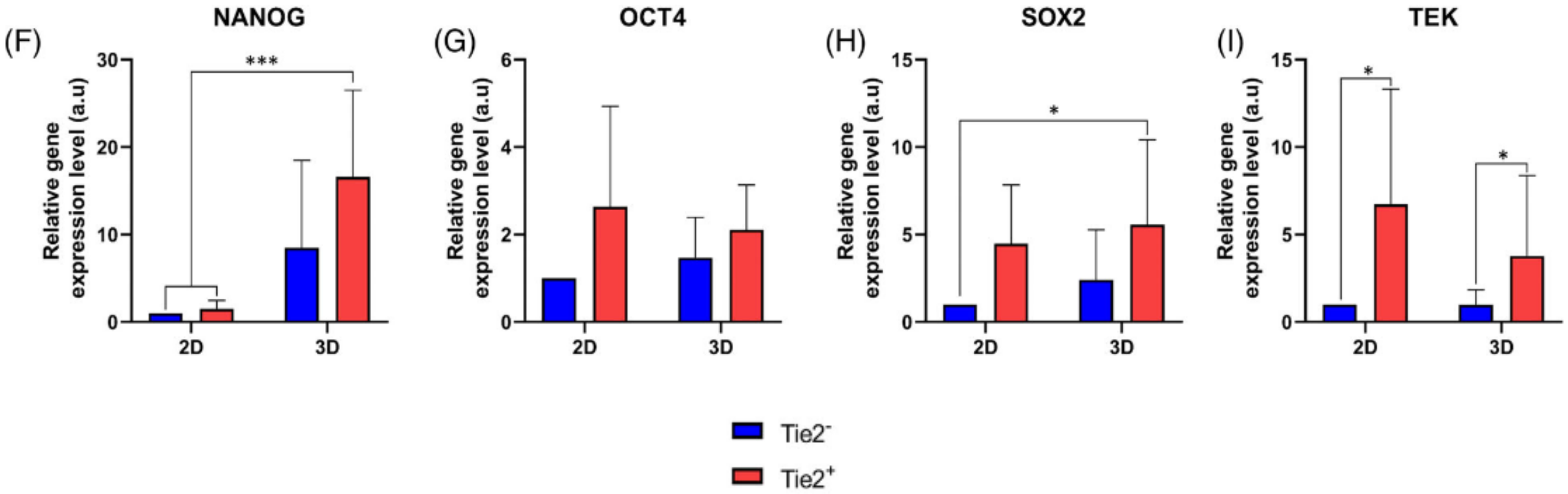




Phase 4 :
Tri-lineage differentiation (ADIPO, OSTEO and CHONDRO) of hBMSCs, NP cells cultured in 2D and NP cells cultured in 3D.

Results

Tie2⁺ versus Tie2⁻ NP cells



NP cells (Tie2⁺ or Tie2⁻) cultured in 2D vs 3D, at the end of Phase 3.

Discussion

- **Novel** and **efficient two-phase expansion culture method** for **human NPCs.**
- **3D culture** was better **mimicking the microenvironment** of the NP tissue inducing a **higher percentage of Tie2** expressing cells.
- **3D configuration** of NPCs allowed to keep the **expression of pluripotent genes** like NANOG, SOX2, and OCT4, or the expression of the TEK gene.
- **3D culture** used in this study **maintained the NP phenotype**, as confirmed by the expression patterns of surface markers (**Tie2** and **CD34**).
- **3D culture** allowed the differentiation into **adipo-**, **chondro-** and **osteo-genic** lineage.

Summary points

- We established a **novel** and **efficient primary culture** and expansion culture method for **human NPCs** consisting of sequential primary 3D alginate culture with **ascorbic acid** supplemented medium followed by 2D monolayer culture on **fibronectin-coated dishes containing FGF-2**.
- This protocol shows that heterogenic NP cell populations are closer to a multipotent phenotype if cultured in 3D within alginate beads compared to 2D culture.
- Moreover, NPCs were able to better differentiate into **osteogenic**, **chondrogenic**, and to a lesser extent **adipogenic** lineage even after *in vitro* expansion than 2D monolayer expanded NPCs.
- As we experimented with pure $Tie2^{+}$ and $Tie2^{-}$ cell populations, it is highly suggested that the **maintenance of multipotent capacity** was mainly but not exclusively due to the **higher presence of $Tie2^{+}$ cells** in the 3D culture.