5. Abstract

Stem cell based Intervertebral Disc Regeneration – Evaluation in Organ Culture

**Background:** The fate of human mesenchymal stem cells (hMSCs) supplied to degenerating intervertebral disc (IVD) is still not fully understood and can be negatively affected by the low oxygen, pH and glucose concentration of the IVD environment. hMSC survival and yield upon injection of compromised IVD could be improved by the use of an appropriate carrier and/or by predifferentiation of hMSCs prior to injection.

**Purpose:** Optimize hMSC culture conditions in thermoreversible hyaluronan-based hydrogel (HA-pNIPAM) to achieve differentiation toward the disc phenotype *in vitro* and evaluate whether preconditioning contributes to a better hMSC response *ex-vivo*.

**Study design:** *In vitro* and *ex-vivo* whole organ culture of hMSCs.

**Methods:** hMSC *in vitro* cultures were conducted in HA-pNIPAM and alginate for one week under hypoxia in chondropermissive medium alone and with the supplementation of transforming growth factor β1 (TGF-β1) or growth and differentiation factor 5 (GDF-5). *Ex-vivo*, hMSCs were either suspended in HA-pNIPAM and directly supplied to the IVDs or pre-differentiated with GDF-5 for one week in HA-pNIPAM and then supplied to the IVDs. Cell viability was evaluated by Live-Dead assay and DNA, GAG and gene expression profiles were used to assess hMSC differentiation toward the disc phenotype.

**Results:** HA-pNIPAM induced hMSC differentiation toward the disc phenotype more effectively than alginate. *In vitro*, higher GAG/DNA ratio and higher COL2, SOX9, KRT19, CD24 and FOXF1 expression were found for hMSCs cultured in HA-pNIPAM compared to alginate, regardless of the addition of growth factor. *Ex vivo*, direct combination of HA-pNIPAM with the disc environment induced a stronger disc-like differentiation of hMSCs than predifferentiation of hMSCs prior to supply to the disc.

**Conclusions:** Hyaluronan-based thermoreversible hydrogel supports hMSC differentiation toward the disc phenotype without the need growth factor supplementation both *in vitro* and *ex-vivo*. This hydrogel may provide an effective stem cell carrier for the treatment of intervertebral disc degeneration.

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