Zurich University of Applied Sciences
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Institute of Chemistry and Biological Chemistry

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# Cell-seeded thermoreversible hydrogel-polyurethane composites for nucleus pulposus augmentation

[1,3]Striegl B, [3]Grad S, [3]Eglin D, [2]Benneker L, [3]Peroglio M, [3]Alini M, [1]Graf-Hausner U, [1]Bono E

1 Zurich University of Applied Sciences, Waedenswil, Switzerland,
2 Insel Hospital, Bern, Switzerland, 3 AO Research Institute, Davos, Switzerland

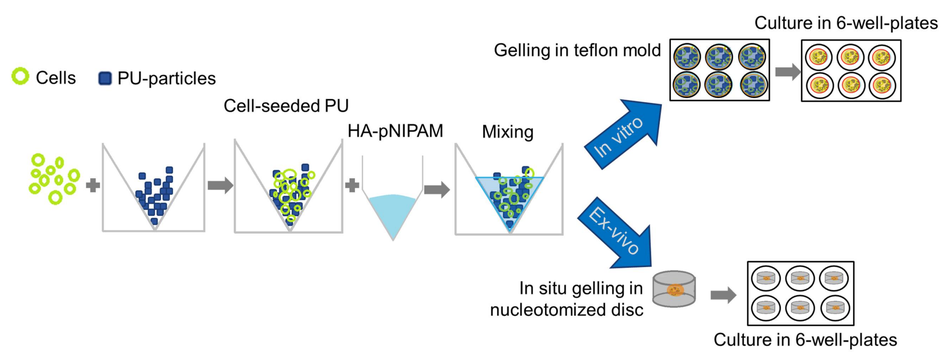
## Introduction

Tissue engineering represents an alternative approach to the current invasive surgical procedures for the intervertebral disc (IVD) repair. The combination of injectable hydrogels and elastomeric biomaterials allows three-dimensional cell cultures and provides mechanical stability. In the present study a thermoreversible hyaluronan (HA) hydrogel as well as fibrin glue were mixed with polyurethane (PU) particles and their effect was investigated on the proliferation and differentiation of human IVD (hIVD cells) and mesenchymal stem cells (hMSCs) by *in vitro* and *ex-vivo* experiments.

## Methods

***In vitro* study:** hMSCs or hIVD cells were seeded on PU particles and embedded in hyaluronan-poly(N-isopropylacrylamide) (HA-pNIPAM)[1,2] or fibrin hydrogel[3]. As controls cells seeded- hydrogels and PU were prepared. All samples were cultured in chondrogenic medium for 3 and 7 days and analyzed for DNA, glycosaminoglycans (GAG), mRNA expression and histology (3 independent experiments in triplicates per condition).

***Ex-vivo* study:** hMSCs seeded-PU particles were embedded in HA-pNIPAM and used to fill bovine nucleotomized caudal discs inclusive of endplates. Samples were cultured in chondrogenic medium under perfusion. Samples were harvested after 3 and 7 days and analyzed for cell viability, DNA, GAG and mRNA expression. Two independent experiments (2-3 replicas each) were performed.




**Scheme of the procedure used for the preparation of PU/hydrogel composites**

## Results

***In vitro* study:**

- hMSCs were able to proliferate in PU/HA-pNIPAM as well as in PU/fibrin samples with the highest DNA amount in the PU/fibrin condition after one week. A lower cell proliferation characterized all HA-pNIPAM samples. hIVD cells showed a similar DNA profile.
- hMSC-seeded PU/HA-pNIPAM samples showed a significant increase of GAG amount vs all other groups. hIVD cells produced significantly less GAG in the PU/HA-pNIPAM condition compared to hMSCs. Moreover, the GAG retention (ratio of GAG in sample to the sum of GAG in the sample and GAG released in the medium) at day 7 was 50-80% for hMSC and 20-60% for hIVD groups (fig.1).
- hIVD cell redifferentiation was confirmed by the gene expression pattern with a strong up-regulation of collagen type II and, to a lower extent, of aggrecan and SOX9 without significant differences between the two gels (fig.2).

***Ex-vivo* study:**

- hMSCs maintained a high viability after implantation in bovine IVDs.
- A slight decrease of DNA and GAG was found over the culture period, possibly due to a loss of hydrogel structure cohesion.
- The IVD environment supported hMSC differentiation toward the NP phenotype (fig.3).

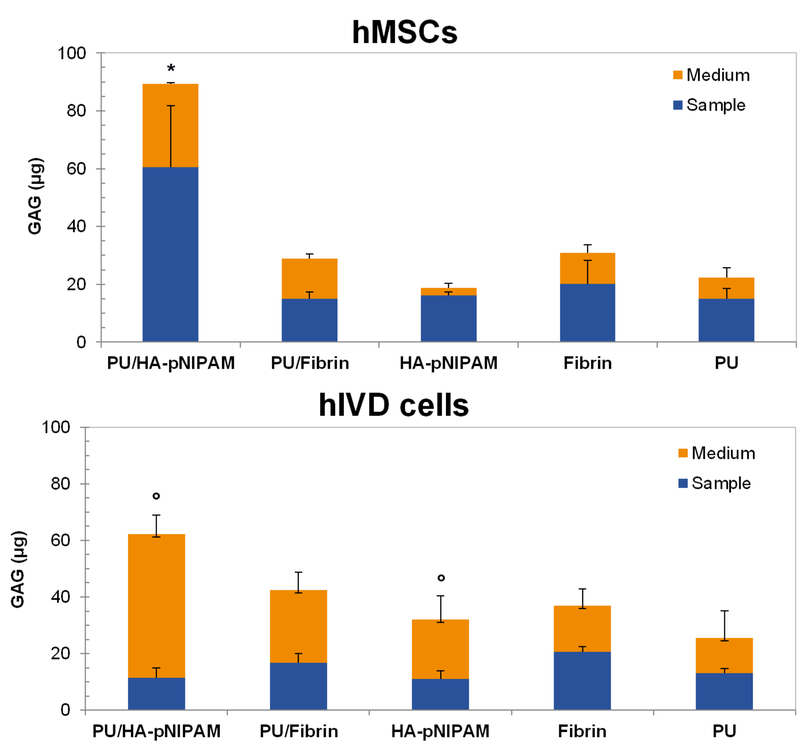



**Fig. 1** GAG content in the samples and released in the medium at day 7 (n=9)

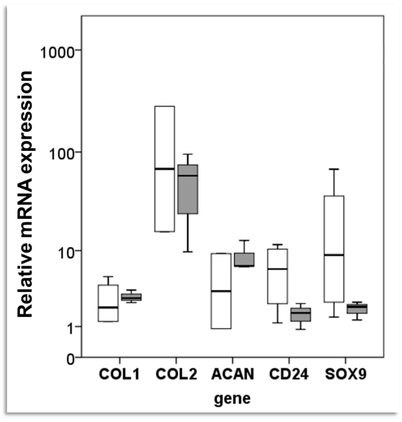



**Fig. 2** Gene expression pattern of hIVD cells cultured in PU/HA-pNIPAM hydrogel (white) and PU/fibrin control (grey). Data are expressed relative to day 3 of culture for each material (n=5).

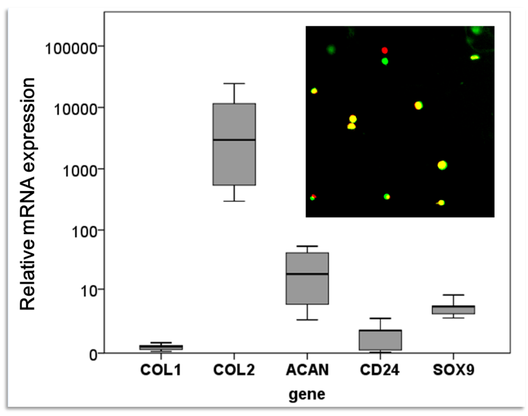



**Fig. 3** Gene expression profile of hMSCs after 1 week of culture in nucleotomized bovine IVDs (n=5). Data are expressed relative to cells collected on the day of seeding. Insert: Calcein AM staining of red PKH26 labelled hMSCs after 7 days ex-vivo (yellow=live hMSCs, red=dead hMSCs).

## Conclusions

- hMSCs synthesized more GAG than hIVD cells in PU/HA-pNIPAM, indicating that hMSCs may represent a suitable cell type for IVD repair.
- The HA hydrogel association with PU particles seems to be a promising strategy for MSC differentiation and may represent a potential candidate for the nucleus pulposus restoration.
- *In vitro* as well as *ex-vivo* studies demonstrated that cells acquire an NP phenotype in the PU/hydrogel composites cultured in chondrogenic medium.

### References

1. Mortisen D et al. Biomacromolecules 2010;11:1261-72. 2. Peroglio M et al. Eur Spine J, Epub ahead of print. 3. Mauth C et al. Eur Cell Mater 2009;18:27-38