A Quick Potency Assay for Osteogenic and Chondrogenic Differentiation and Evaluation of Donor Variability of Adipose Derived Stem Cells

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Introduction

Osteogenic and chondrogenic differentiation potential of adipose derived stem cells (ASCs) are promising for bone- and cartilage repair in tissue engineering. Large donor variability requires testing of the differentiation potential prior to implantation. Current methods to analyze the differentiation capacity are time consuming and thus we focus in this project on the development of fast and sensitive bioassays with our novel tissue-specific and signal-amplified promoter (1).

Discussion & Conclusion

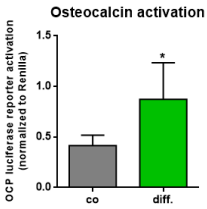
We designed and established novel enhancer and tissue-specific promoter for osteogenic and chondrogenic differentiation of ASCs together with a quick potency bioassay. Although the lineage specific markers osteocalcin and collagen type II are expressed at late timepoints, with our sensitive gene assay the activation of the signal-amplified reportergenes can already be determined after 3 days of differentiation *in vitro*. In conclusion osteocalcin and collagen type II might be useful reportergenes for a fast detection of ASC differentiation and donor variability.

OSTEOGENESIS

day 3

day 21

Fig. 1. a.



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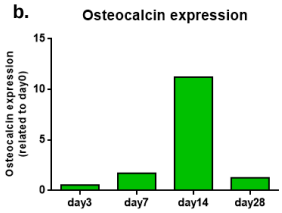

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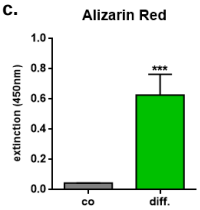

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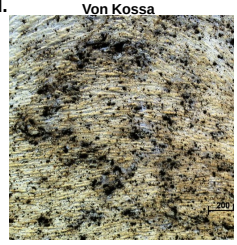

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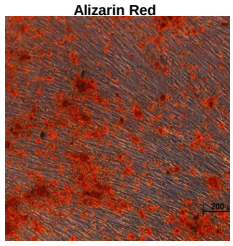
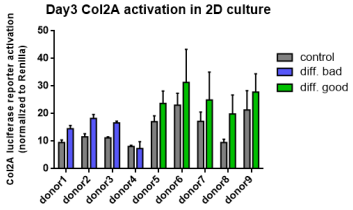


CHONDROGENESIS

day 3

day 35

Fig. 2. a.



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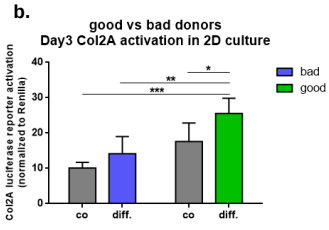

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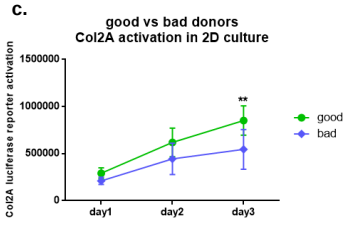

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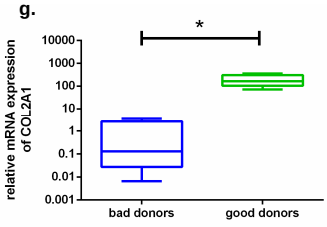

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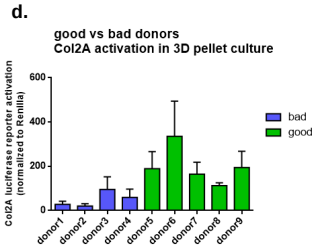

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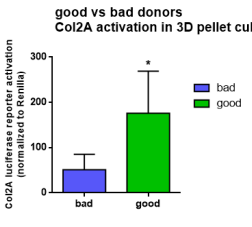

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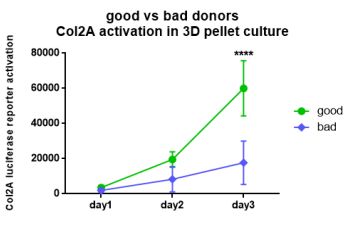

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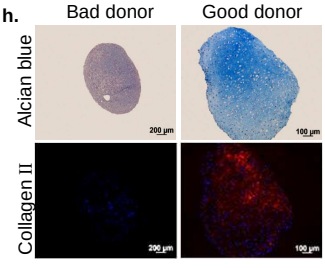

Material & Methods

Human primary ASCs were co-transfected with luciferase based reportergenes pCMVE/mOCP-MetLuc (osteocalcin) and pCMVE_ACDCII-MetLuc (collagen type II) together with renilla control plasmid by lipofection. Luciferase activities were compared to standard osteogenic- and chondrogenic differentiation assays such as von Kossa and Alizarin red, or collagen type II staining. Furthermore the reportergene expression was correlated to tissue specific gene expression.

Acknowledgements: The authors thank Ludwig Aigner from Paracelsus Medical University, Salzburg for providing renilla control plasmid.
Literature: (1) Feichtinger GA, et al. Tissue Engineering 17, 4, 2011.
Statistics: Students t-test n=4-5, one-way ANOVA Tukey's post hoc, two-way ANOVA Bonferroni post-hoc (p 0.0001 - 0.05), n=9

Results

OSTEOGENESIS: The activity of the reportergene pCMVE/mOCP-MetLuc was enhanced in transfected ASCs treated with osteogenic differentiation media compared to control condition (Fig.1a.) Osteogenic differentiation was confirmed with Alizarin red quantification (Fig.1c.), von Kossa (Fig.1d) and Alizarin red histological staining (Fig.1e). These findings were further verified by qRT-PCR for osteocalcin (Fig.1b).
CHONDROGENESIS: The activity of the reportergene pCMVE_ACDCII-MetLuc was measured in selected good and bad donors in 2D-culture (Fig.2a.-c.) and 3D-culture (Fig.d.-f.). Chondrogenic differentiation was confirmed with collagen type II and Alcian blue staining (Fig.2h.). These findings were further verified by qRT-PCR (Fig.2g). 2D as well as 3D pellet culture showed significant induction of collagen type II in good donors.