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Intraindividual comparison of the osteogenic differentiation potential of mesenchymal stem cells derived from adult adipose tissue and bone marrow

Intraindividual comparison of osteogenic differentiation potential of ATSC and BMSC

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Introduction

Adipose tissue, like bone marrow (BMSC), is a promising source to recover multipotent mesenchymal stromal cells (ATSC) for tissue enginnering and regeneration of bone. FACS-analyses even show a specific marker profile for ATSC, like for BMSC. Osteogenic differentiation of BMSC and ATSC is conventionally mediated by ascorbic acid, dihydroxycholecalciferol and dexamethasone/ß-glycerophosphate (conventional conditions) and by the addition of rhBMP-2 in 2D- and 3D-cell culture systems (collagenous scaffolds).

Objectives

The lack of sources for autologous bone transplantation has led to the development of different strategies for bone replacement. The long-term objective of our work is the establishment of bone regeneration strategies for the treatment of osseous defects after trauma or ablative tumor surgery by tissue engineering. It is considered, that cure of osseous defects is even ideally approachable to regenerative strategies.

Material and Methods

Human adipose tissue (AT) and bone marrow (BM) were obtained from 4 patients and mesenchymal stromal cells were isolated and analysed (FACS, n=14).
Osteogenic differentiation under conventional conditions was compared to the addition of rhBMP-2 (10mg/ml). Histological stainings were prepared of 2D- and 3D-cell culture systems. RNA-expression of osteocalcin (OC), osteopontin (OP), osteonectin (ON) and alkaline phosphatase (AP) was analysed quantitative by RT-PCR (LightCycler) in 2D-cultures.

Results

1. Osteogenic differentiation of ATSC in vitro:

Isolated ATSC show the same marker-profile expression (CD29+, CD31-, CD34-, CD44+, CD73+, CD90+, CD105+, CD133-, CD166-) like BMSC in FACS-analyses (Fig.1).
In 2D- and 3D-cell culture systems in vitro (Fig. 2) the osteogenic differentiation of ATSC could be shown by the detection of OC-producing cells (immunhistological staining) in the mineralisation areas (v.Kossa).
3D-cell culture systems in a SCID-mouse model show in vivo (Fig. 3) even a osteogenic differentiation of ATSC in immunhistological stainng of OC and trabecular bone formation.

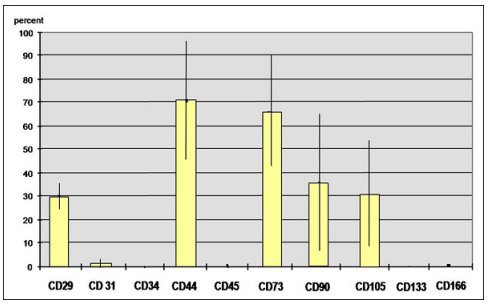


Fig. 1: FACS-analyses (n=14)

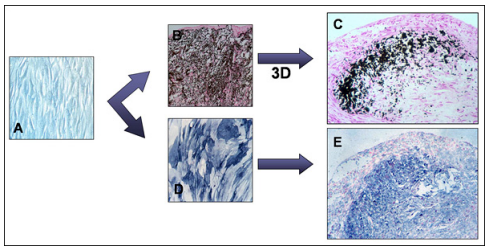


Fig. 2: Histological staining of osteogenic differentiated ATSC (A) with mineralisation areas (v.-Kossa, B/C), and osteocalcin-expression (D/E)

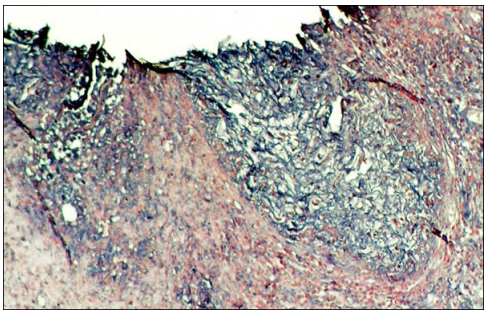


Fig. 3a: Imunhistological staining of OC

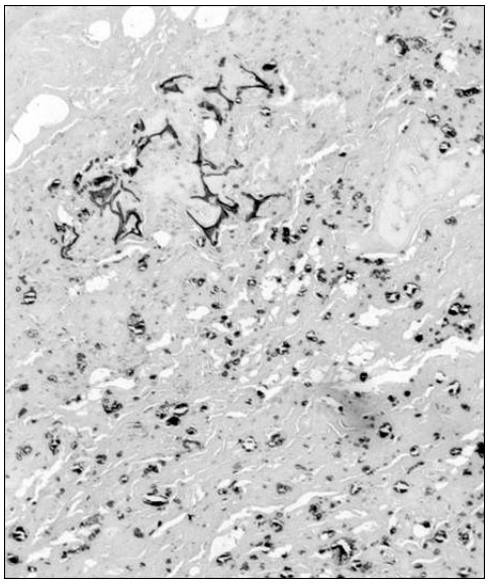
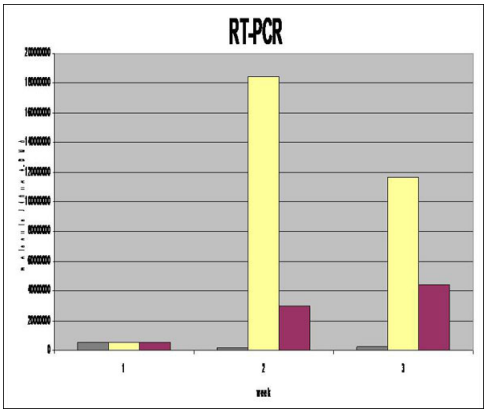


Fig. 3b: Imunhistological staining of HE

2. Comparison of the osteogenic differentiation potential of ATSC/BMSC:

Osteogenic differentiated ATSC and BMSC showed under conventional differentiation conditions a high expression of osteocalcin (OC) in FACS-analysis and RT-PCR (relative: ATSC > BMSC: 15:1, Fig.4).



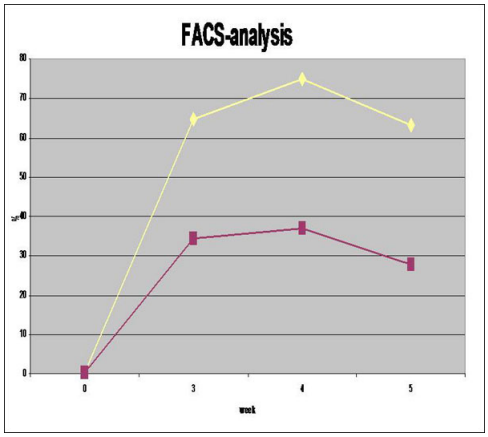


Fig. 4a-c: RT-PCR and FACS-analysis of OC

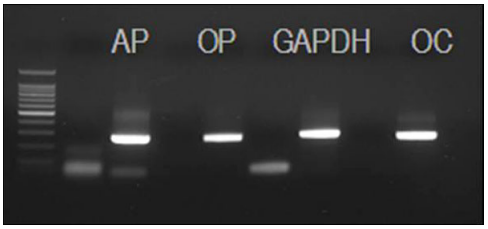


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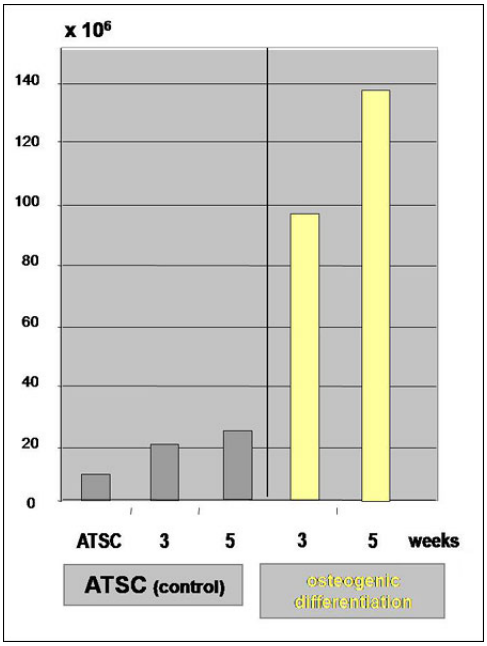
Fig. 4a-c: RT-PCR and FACS-analysis of OC

AP-expression incresed during the whole differentiation period in ATSC, while ON-expression (active remodeling) increased clearly in the 5th week of osteogenic differentaition in BMSC while OPN-expression (tissue integrity) decreased during the differentiation in both (ATSC/BMSC, Fig.5).
By application of rhBMP-2 was only an increase of the ON- and OPN-expression in BMSC during the differentiation rhBMP-2 period in shown (Fig.6).



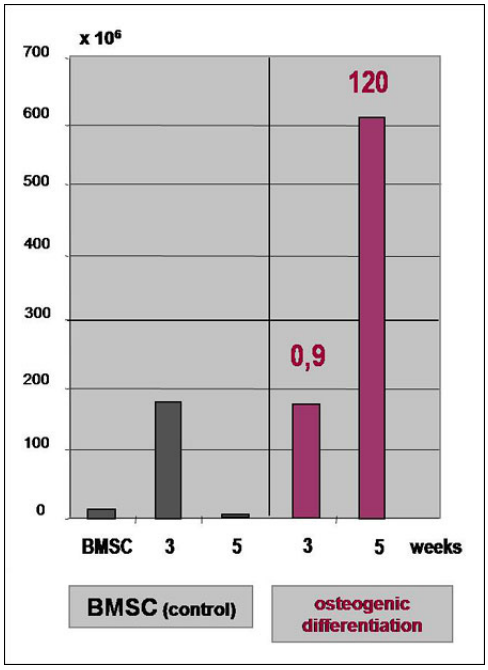
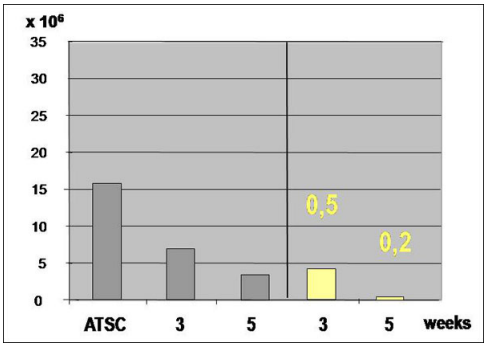
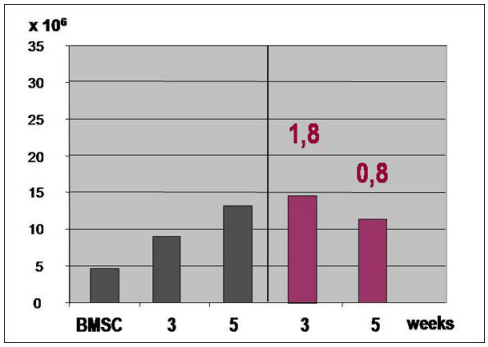


Fig. 5a-d: RT-PCR of AP-, ON-and OPN-expression





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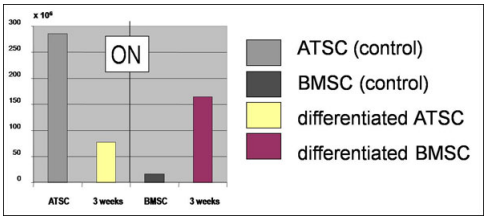

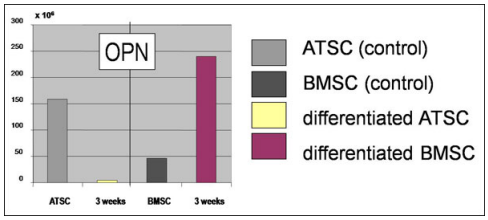

Fig. 6a-b: ON-and OPN-expression under rhBMP2-application during differentiation

Conclusions

Osteogenic differentiation is possible in ATSC and BMSC (different potential) and showed in ATSC a lower osteogenic potential than in BMSC. The osteogenic differentiation potential of rhBMP-2 in our protocol is less effective

Literature

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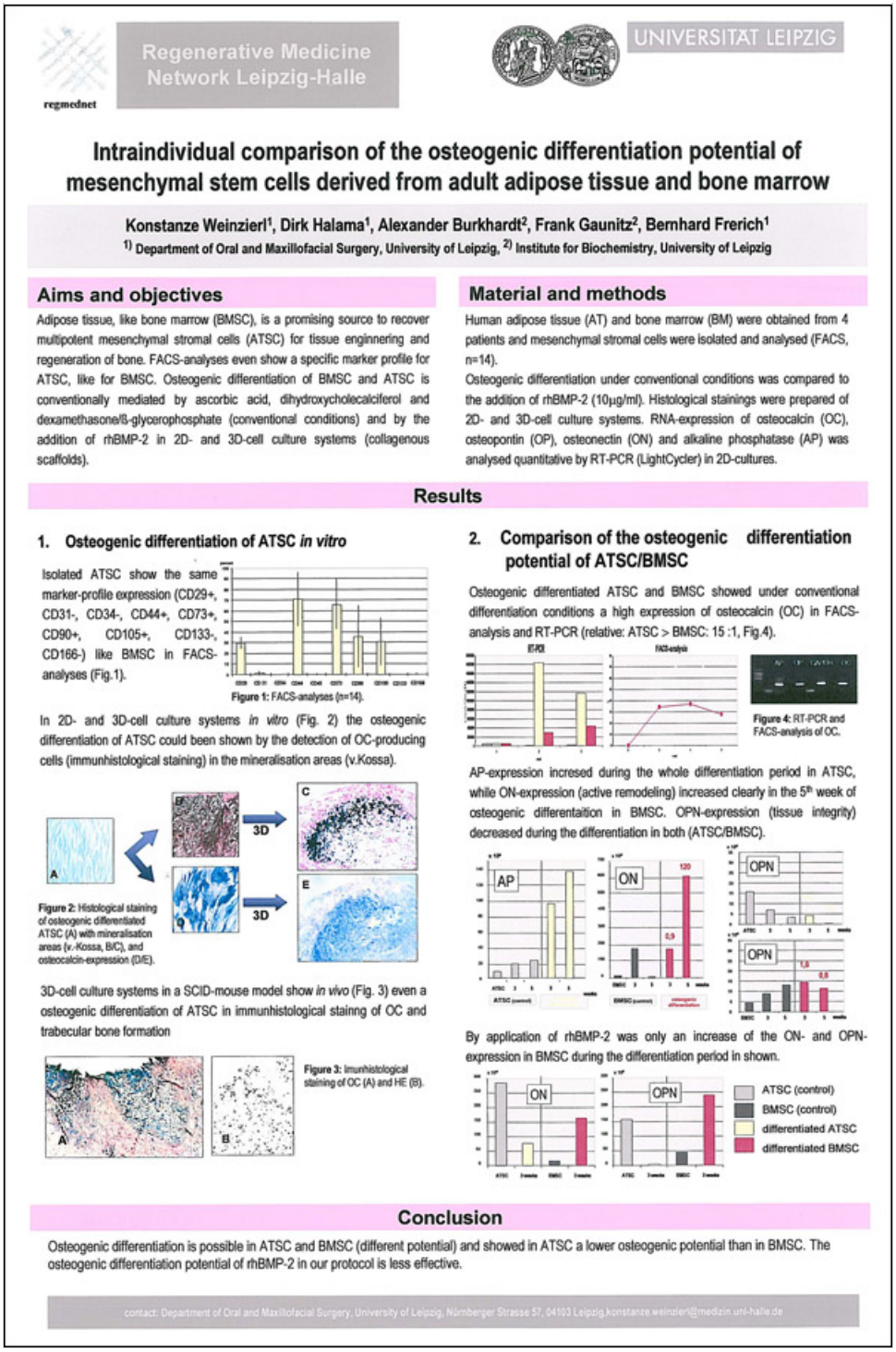

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Intraindividual comparison of the osteogenic differentiation potential of mesenchymal stem cells derived from adult adipose tissue and bone marrow

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Aims and objectives

Adipose tissue, like bone marrow (BMSC), is a promising source to recover multipotent mesenchymal stromal cells (ATSC) for tissue enginnering and regeneration of bone. FACS-analyses even show a specific marker profile for ATSC, like for BMSC. Osteogenic differentiation of BMSC and ATSC is conventionally mediated by ascorbic acid, dihydroxycholecalciferol and dexamethasone/ß-glycerophosphate (conventional conditions) and by the addition of rhBMP-2 in 2D- and 3D-cell culture systems (collagenous scaffolds).

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Figure 1: FACS-analyses (n=14).

In 2D- and 3D-cell culture systems *in vitro* (Fig. 2) the osteogenic differentiation of ATSC could been shown by the detection of OC-producing cells (immunhistological staining) in the mineralisation areas (v.Kossa).

Figure 2: Histological staining of osteogenic differentiated ATSC (A) with mineralisation areas (v.-Kossa, B/C), and osteocalcin-expression (D/E).

3D-cell culture systems in a SCID-mouse model show *in vivo* (Fig. 3) even a osteogenic differentiation of ATSC in immunhistological stainng of OC and trabecular bone formation

Figure 3: Imunhistological staining of OC (A) and HE (B).

2. Comparison of the osteogenic differentiation potential of ATSC/BMSC

Osteogenic differentiated ATSC and BMSC showed under conventional differentiation conditions a high expression of osteocalcin (OC) in FACS-analysis and RT-PCR (relative: ATSC > BMSC: 15 :1, Fig.4).

Figure 4: RT-PCR and FACS-analysis of OC.

AP-expression incresed during the whole differentiation period in ATSC, while ON-expression (active remodeling) increased clearly in the 5th week of osteogenic differentaition in BMSC. OPN-expression (tissue integrity) decreased during the differentiation in both (ATSC/BMSC).

By application of rhBMP-2 was only an increase of the ON- and OPN-expression in BMSC during the differentiation period in shown.

ATSC (control)
BMSC (control)
differentiated ATSC
differentiated BMSC

Conclusion

Osteogenic differentiation is possible in ATSC and BMSC (different potential) and showed in ATSC a lower osteogenic potential than in BMSC. The osteogenic differentiation potential of rhBMP-2 in our protocol is less effective.

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