Stromal derived factor-1 stimulates cell recruitment and vascularization in an ectopic bone forming model

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Introduction:
Improvement of functional large bone tissue constructs remains a challenge in regenerative medicine. Autologous bone is often used, but alternatives are needed due to several disadvantages. By using bioactive materials as a carrier, for example, for cells or growth factors, bone formation can be induced. In addition, usage of hydrogels has a great potential as drug delivery systems for several bone inducing agents. The chemokine stromal derived factor-1α (SDF-1α) is known to stimulate recruitment of injured tissues by recruitment of endogenous cells. SDF-1α is also known to induce vasculogenesis, an essential event prior to bone formation. In the present study, we used a hydrogel based construct to obtain local delivery of SDF-1α. Hereby, we aim to recruit cells to ectopic bone forming sites and stimulate vascularization and osteogenesis.

Methods:
Constructs consisting of 200µl Matrigel (BD Biosciences) plugs supplemented with 200ng/ml recombinant murine SDF-1α (R&D Systems), were implanted subcutaneously in nude mice (n=9). As controls, empty Matrigel plugs or Matrigel plugs seeded with isolated mouse multipotent stromal cells (MSCs, 10⁶ cells/ml) were used; each mouse received all constructs. All constructs contained 20% of biphasic calcium phosphate (bcp) particles of 100-200 µm diameter. Implants were retrieved after 1 week (n=3) and 6 weeks (n=6). All implants were decalcified, processed for paraffin embedding and stained with haematoxylin and eosin. Sections were evaluated for cell recruitment and the formation of vessel-like structures. Week 6 implants were also evaluated for osteogenic differentiation by osteocalcin immunohistochemistry. Statistical analysis was performed by one-way ANOVA and Bonferroni correction was applied; p-values of <0.05 were considered statistically significant. Data are expressed as mean ± standard deviation.

Results:
The empty control constructs showed hardly any recruitment of endogenous cells or the formation of vessel-like structures after 1 week of implantation (Fig. 1A). In contrast, SDF-1α constructs showed some endogenous cell recruitment throughout the constructs after one week, as well as formation of vessel-like structures, some of which were perfused as evidenced by presence of erythrocytes (Fig. 1B). After six weeks, endogenous cells were observed in empty constructs (Fig. 1C), whereas SDF-1α constructs showed abundant recruitment of endogenous cells (Fig. 1D), which was significantly different compared to empty constructs (Fig. 2A). Vessel-like structures were abundantly present after six weeks in SDF-1α constructs (Fig. 1D), although not significantly different from the control constructs (Fig. 2B) at this timepoint. The constructs that were seeded with mouse MSCs (Fig. 1E) showed similar degrees of cell recruitment and vessel-like structure formation as SDF-1α constructs (Fig. 1D). Quantifications showed no significant differences between these two groups for both parameters (Fig. 2A and 2B). Osteogenic differentiation of cells present inside the constructs could be observed in all constructs after six weeks, as shown by osteocalcin staining (Fig. 2A-C).

Discussion:
Here, we demonstrate that local presence of SDF-1α in vivo results to increased endogenous cell recruitment inside SDF-1α hydrogels after one week. This effect was long lasting since we found a significant difference after six weeks between empty and SDF-1α constructs. In addition, usage of SDF-1α hydrogels showed increased formation of vessel-like structures, many of which were blood perfused. The recruited cells were able to differentiate towards the osteogenic lineage to a similar degree in all constructs after six weeks. No bone formation was found yet. This may be explained by the relatively low concentration SDF-1α used in this study. In addition, we expect a delay due to the slow hydrogel breakdown. In conclusion, we show for the first time a model to recruit endogenous cells in order to stimulate vascularization and osteogenic differentiation with a single chemokine at specific locations.

Significance:
Usage of SDF-1α laden hydrogels enables us to deliver this chemokine locally and induce cell recruitment and vessel formation at the site of interest. Optimizing drug-laden hydrogels could result in cell free constructs for future use in bone regeneration.

Figure 1. Representative pictures of SDF-1α constructs (B, D) showing increased cell numbers (arrows) and vessel (v) formation compared to empty constructs (A, C), at 1 week (A-B; n=3) and 6 weeks (C-D; n=6). SDF-1α constructs (D) and constructs seeded with mouse MSCs (E; n=6) showed comparable cell numbers m: Matrigel; bcp: biphasic calcium phosphate

Figure 2. SDF-1α constructs showed increased cell presence (A) and vessel-like structure formation (B) inside the constructs after six weeks (n=6). **p<0.01 compared empty constructs.

Figure 3. Osteogenic differentiation occurred in empty (A), SDF-1α constructs (B) and constructs seeded with mouse MSCs (C), n=6; arrows indicate positive osteocalcin staining; bcp: biphasic calcium phosphate

References:
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