Intermittent Parathyroid hormone treatment reduces scar tissue formation at the proximity of calvarial grafts, demonstrate by collagen-sensitive MRI scanning methods

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Target audience

We are presenting an innovation to the MRI field, targeted to researchers and clinicians interested in the phenomenon of fibrotic scar tissue formation and its quantification.

Purpose

Bone loss in the craniofacial complex due to trauma or disease presents a major clinical challenge. Bone allografts composed of nonvital bone serve as a widely accepted solution - though often fail to integrate with host bone due to formation of fibrotic scar tissue. We have recently showed that daily short-time administration of parathyroid hormone (PTH) following allograft implantation promotes stem cell recruitment and differentiation, thereby inducing bone formation with improved osteo-integration at the implantation site[1]. We observed that PTH significantly down-regulates the pro-fibrogenic gene CCN2 of endogenous cells at the graft-host junction. To further examine the PTH effect on scar tissue formation by molecular and imaging approach, we have developed a unique MRI scanning protocol to measure collagen fiber deposition in the scar-bone suture area. **We hypothesize that this quantitative MRI technique for evaluating collagen deposition will confirm that PTH administration decreases collagenous scar tissue formation.**

Methods

Circular calvarial bone defects (5 mm in diameter) were created in FVB/n mice. The mice were implanted with decellularized allografts, with or without daily PTH treatment (40 μg/kg/day). The animals were euthanized four weeks after surgery and the calvarial region isolated. Half of the ex-vivo samples were kept frozen in -80°C for no more then a week and half were scanned immediately with 360MHz microscopic MRI (μMRI) – using magnetization transfer contrast (MTC) and MEX protocols[2] based on direct measuring of magnetization transfer rates between water and collagen. These methods were found, in previous studies, to be sensitive to tissue organization and to the amount of collagen. The μMRI scans were followed by a micro-computed tomography (μCT) scan and histological analysis.

Results

Ex-vivo MRI scans of fresh samples harvested from PTH treated animals, using the MTC protocol, showed significantly higher magnetization transfer ratio (MTR) comparing to the control animals. T2 values of the both experimental groups were similar, suggesting that the enhancement can be attributed to changes in collagen. Interestingly, when frozen samples were scanned these results were not reproduced. MEX μMRI scans of the PTH samples showed slower signal building along time, comparing to the control samples. μCT scans ensured that the μMRI signal correlates to the pertinent anatomical site and demonstrated new bone formation at the allograft-host interface in the PTH-treated group. The presence of collagen fibers at the graft-host junction was confirmed by histological analysis.

Discussion

The significant enhancement of the ex-vivo MTR signal indicates augmented deposition of rigid collagen fibrils and fibers in PTH treated animals. We found that sample freezing damages the soft collagenous tissues or alter the water quantity at the graft surroundings resulting in mixed results. The curves of MEX signal measured in the control animals hardly showed buildup, in contrast to the PTH treated animals. This reflects higher rate of magnetization exchange for the former samples. Both methods, MTC and MEX, gave clear images of the scar tissues at the graft host suture. While MTC had the advantage of better SNR and probably more sensitive to the amount collagen, the MEX gave information about the extent of the bone mineralization. These results can be attributed to the presence of inorganic ions in the PTH treated samples, ions assembling the calcifying bone mineral. Thus, the proposed approaches enable differentiation between new bone formation and scar tissue. The enhanced new bone formation demonstrated by the μCT scan was possible due to the decrease in scar tissue formation.

Conclusion

The approaches presented in the current study will enable physicians to assess the clinical outcome of surgical procedures intended to treat non-union fractures in a non-invasive manner as well as monitor a spectrum of other inflammatory conditions involving scar tissue formation, such as cirrhosis, renal glomerular fibrosis, asthma, and various autoimmune diseases.

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References

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