Selective Targeting of Bone and Cartilage with Multivalent Dendritic Polyanions

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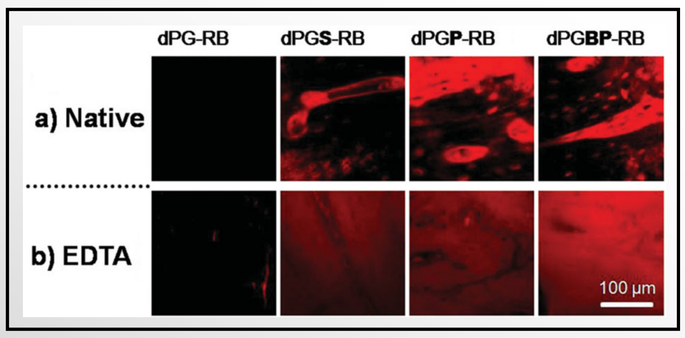
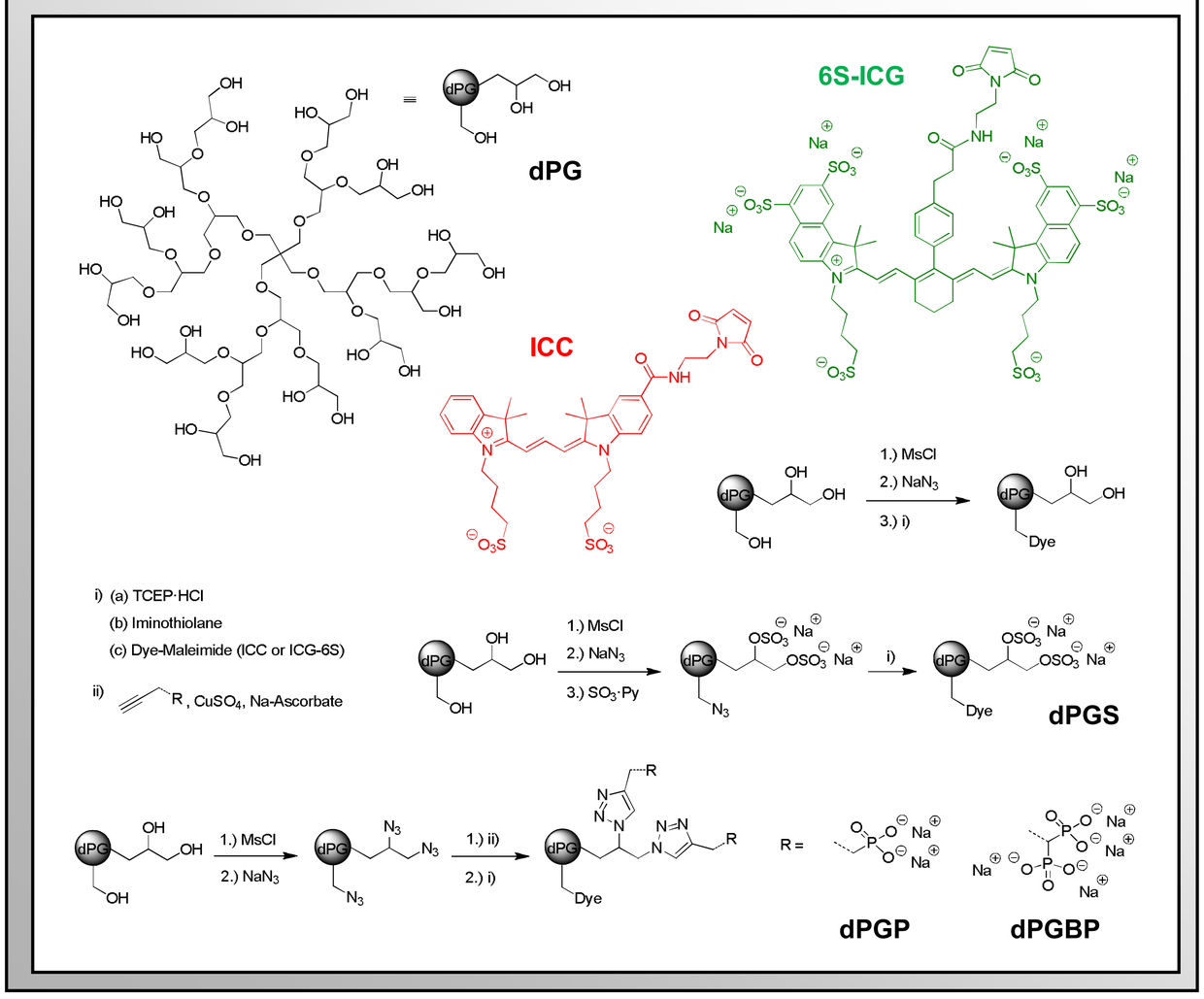
Introduction

In previous studies native and demineralized ovine bone was used to analyze the affinity of polyanions derived from dendritic polyglycerol (dPG) toward hydroxyapatite and collagen.[1] Whereas the neutral polymer did not show any interaction with bone, a selective binding to hydroxyapatite and collagen was observed depending on the anionic moiety of the polymer. Based on these results the binding affinity of polyanions toward cartilage was investigated to obtain selective targeting agents.



Synthesis of Dye-Labeled Polyanions



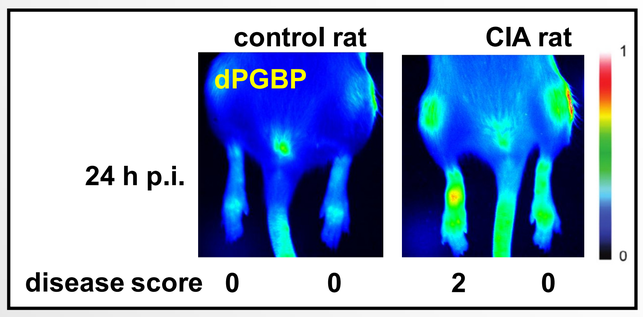
Methods

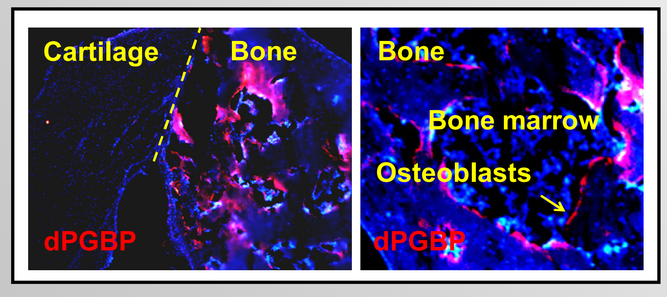
Rheumatoid arthritis was induced in female Lewis rats (150 ± 10 g) by subcutaneous injection of collagen II after 1 and 8 days. NIR-fluorescence imaging in vivo was performed 24h after one single i.v. injection of the 6S-ICG dye conjugates (2 mg/kg) via the tail vein. Animals included for later histology additionally received ICC conjugates (1 mg/kg). Histological recovery of the dye conjugates was analyzed by fluorescence microscopy. Histochemical staining was performed on cryo sections on cellotape of tibiotarsal articulations.

Sections of freshly explanted porcine cartilage were incubated with Interleukin-1ß (IL-1ß) for 24h. Pictures were taken 24h after incubation with 6S-ICG labeled conjugates (10^{-6} mol L^{-1}) by fluorescence microscopy. For glycosaminoglycan (GAG) release studies similar sized discs of the cartilage explants were incubated with IL-1ß alone or IL-1ß and dPG or dPG anions, respectively for 24h. Afterwards proteinase K was added and the total concentration of GAGs of the supernatant after centrifugation was determined by the dimethyl methylene blue method (DMMB). Untreated cartilage served as control.

Binding Affinity to Joints in Vivo

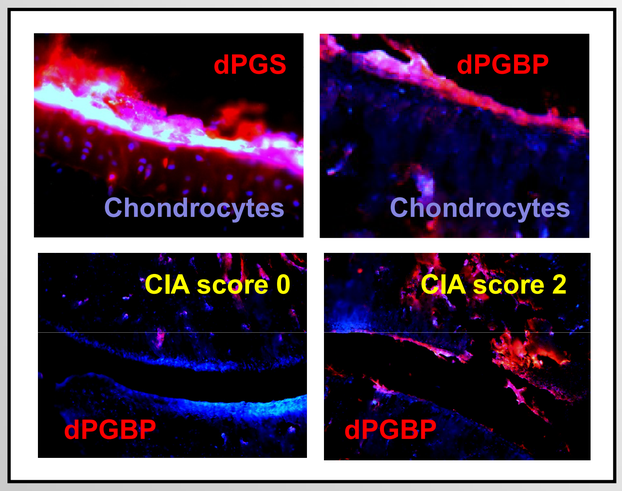
Dendritic polyglycerol sulfate (dPGS) known as anti-inflammatory agent accumulates in inflamed joints in rheumatoid arthritis (CIA). [2] Using the same animal model, bisphosphonate modified dPG (dPGBP) also shows an affinity to inflamed tissue.



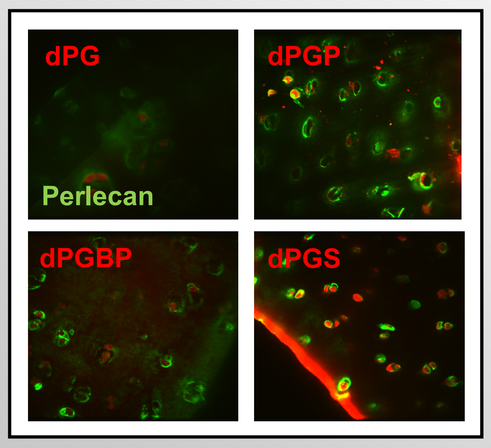


Histological recovery of ICC labeled dPG-BP in healthy rat tissue showed enrichment only in bone, accumulating in osteoblasts.

In CIA rat tissue both dPGS and dPGBP were found to bind to inflamed cartilage.

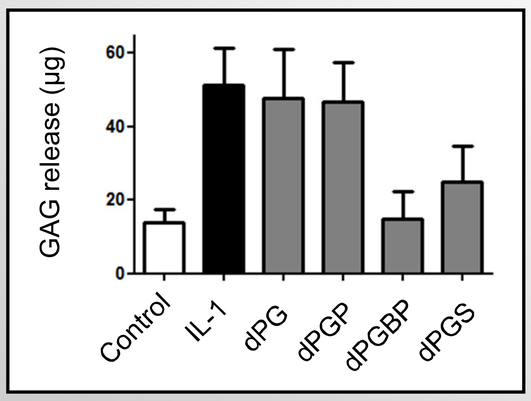


Interaction with IL-1 Treated Explanted Cartilage



Investigating the binding affinity to Interleukin 1 treated porcine cartilage explants, dPGS was found to efficiently penetrate the cartilage tissue and accumulate in chondrocytes.

When investigating the inhibition of glycosaminoglycan (GAG) release dPGS and dPGBP were found to efficiently reduce the GAG loss.



Conclusion

Dendritic polyglycerol based anions were found to interact with bone and cartilage depending on the nature of the anionic group and the disease. dPG anion derivatives are therefore considered as promising candidates for diagnostic and therapeutic applications to target healthy and malfunctioning bone and cartilage.

References

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[2] K. Licha, P. Welker, M. Weinhart, N. Wegner, S. Kern, S. Reichert, I. Gemeinhardt, C. Weissbach, B. Ebert, R. Haag, M. Schirner, *Bioconjugate Chem.* **2011**, *22*, 2453-2460.

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