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The integrity of dermal therapeutic DNAzymes in chitosan polyplexes

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DNAzymes represent a potential new class of nucleic acid-based active pharmaceutical ingredients (API). DNAzymes have the ability to regulate pathological gene expression on a post-transcriptional level by specifically binding to the targeted mRNA through complementary base paring. The bound target is cleaved by the catalytic domain of DNAzymes, resulting in an inhibition of the specific translation [1]. The combination of such specificity and enzymatic activity makes DNAzymes an interesting candidate for the therapy of different diseases.

Some potential DNAzymes are currently being tested for the therapy of skin diseases, including atopic dermatitis, psoriasis and actinic keratosis [2]. To achieve a successful topical treatment, a certain concentration of intact DNAzymes is necessary at the side of action. Therefore, the DNAzymes must be able to penetrate into the first layers of the skin while maintaining their integrity.

Especially during dermal application, DNAzymes are exposed to degrading enzymes. These enzymes are secreted ubiquitously by the skin and resident bacteria on the skin surface. The degradation of potential API reduces the concentration at the site of action and compromises the therapeutic outcome. To counteract degradation, we examined different protective systems for DNAzymes. The most promising approach was the complexation of DNAzymes with chitosan. In this self-assembly process, two polyelectrolytes complexed into polyplexes via electrostatic interaction.

The data of our studies confirmed that the particular DNAzyme could complex to polyplexes. The generation of polyplexes was controlled by the ratio of chitosan's free amino group to DNAzyme's phosphate (N/P ratio). The complexation of DNAzyme consequently reduced the abundance of free DNAzyme. A minimal recovery of less than 3.2 ± 0.2 % of free DNAzyme was achieved. The polyplexes could be decomplexed into basic components by adding hydroxide to the system. The intact DNAzyme could be completely recovered, indicating that the polyplexes did not affect the integrity of the DNAzyme. To validate the protective efficiency of polyplexes, the DNAzyme-chitosan polyplexes were incubated with a degrading deoxyribonuclease. Without protection, and at low N/P ratios, the DNAzyme was nearly completely degraded, while raising the N/P ratio achieved the maximal protective efficiency in our study. In conclusion, polyplexes represent a promising protective system for dermal application of therapeutic DNAzymes.

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2. Schmidts, T.; Marquardt, K.; Schlupp, P.; Dobler, D.; Heinz, F.; Mäder, U. et al. (2012): Development of drug delivery systems for the dermal application of therapeutic DNAzymes. In: Int J Pharm 431 (1-2), S. 61–69.