Supplementary information to the article:

A GMP-compliant formulation of regeneratively active polyphosphate for wound healing and skin regeneration

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Biomarker to measure the superior efficiency of Na-polyP-GMP versus Na-polyP-COM

Only recently, we had Na-polyP-GMP ready for use in wound healing. To assess the differences in healing potency between Na-polyP-COM and the advanced Na-polyP-GMP formulation, we investigated the potency of the effect of both polyP preparations on myofibroblasts. The characteristic feature of myofibroblasts is their contraction property, which is dependent on ATP supply.[1] During the differentiation of fibroblasts to myofibroblasts, the cells elongate and develop a well-developed contractile apparatus based on robust actin stress fibers.[1,2] These stress fibers respond to ATP and have a diameter of 1.0 µm, each and reach lengths of up to 100 µm. Due to the known property of both Na-polyP-GMP and Na-polyP-COM to serve as a source for ATP generation, we measured the length of all the stress fibers in stained sections with myofibroblasts. Of course, the 9 patients treated so far are not enough for regulatory approval of polyP as a medical device.[3-5] However, the formulation of the GMP-conform active ingredient Na-polyP-GMP with the beneficial properties summarized above was a lengthy and arduous process and can be considered as an important step towards an API (Active Pharmaceutical Ingredient) acceptance. The GMP certificate for Na-polyP-GMP was granted by the EMA authority.[2]

The superior efficiency of the Na-polyP-GMP formulation can also be deduced from the present clinical results of the proof-of-concept open studies. It is evident that the mean healing time for all patients studied to date is 6.33 ± 2.06 weeks – a remarkably short period.

Extracellular ATP is a "danger signal" that binds to P2 purinergic receptors (especially on P2Y2 and P2X7) on myofibroblasts.[6,7] This triggers a signaling cascade that involves an increase in intracellular calcium and activation of the RhoA/ROCK pathway, which is the master regulator of actin polymerization and myosin contractility. Extracellular ATP promotes the assembly and stabilization of pre-existing stress fiber components via the RhoA signaling pathway.[8] Already previously, we had proposed that the transition of fibroblasts to myofibroblasts is triggered by ATP, which is produced after enzymatic digestion of polyP with ALP and ADK.[8,9] Therefore, we used the length of the myofibroblasts obtained from sections through the granulation tissue. The length of the stress fibers is correlated with the length and differentiation status of the myofibroblasts.[9-12] , caused by the exogeneous ATP.

For this study, cells obtained from biopsies of chronic wounds of patients, described here not treated with Na-polyP-GMP were compared with those obtained from wounds freshly treated Na-polyP-GMP (after an application period of 12 h). To highlight the different cell morphologies, the same antibody was used for the reaction with human α smooth muscle actin.

However, in the polyP-treated specimens, the immunocomplexes were visualized in red with a goat anti-Alexa Fluor-350 secondary antibody (Fig. S-2 (I-A)), while the immunocomplexes in the polyP-untreated slices were visualized in green with a FITC-labeled secondary antibody (Fig. S-2 (I-B)). In the latter sections, it is evident that the cytoplasm of the fibroblasts also contains a cytoskeleton, which is colored differently due to the actin fibers present in these precursor cells.[9]

For quantification, tissue sections were prepared and analyzed from patients treated with Na-polyP-COM and Na-polyP-GMP. The length of the fibroblasts in the Na-polyP-GMP-treated wound examined here was compared with the length of the cells in the Na-polyP-COM-treated wounds (Fig. S-1). The result showed that the mean size of the myofibroblasts in the Na-polyP-GMP-treated chronic wound was significantly larger than the size of the myofibroblasts in the Na-polyP-COM-treated wounds (Fig. S-2 (II)).

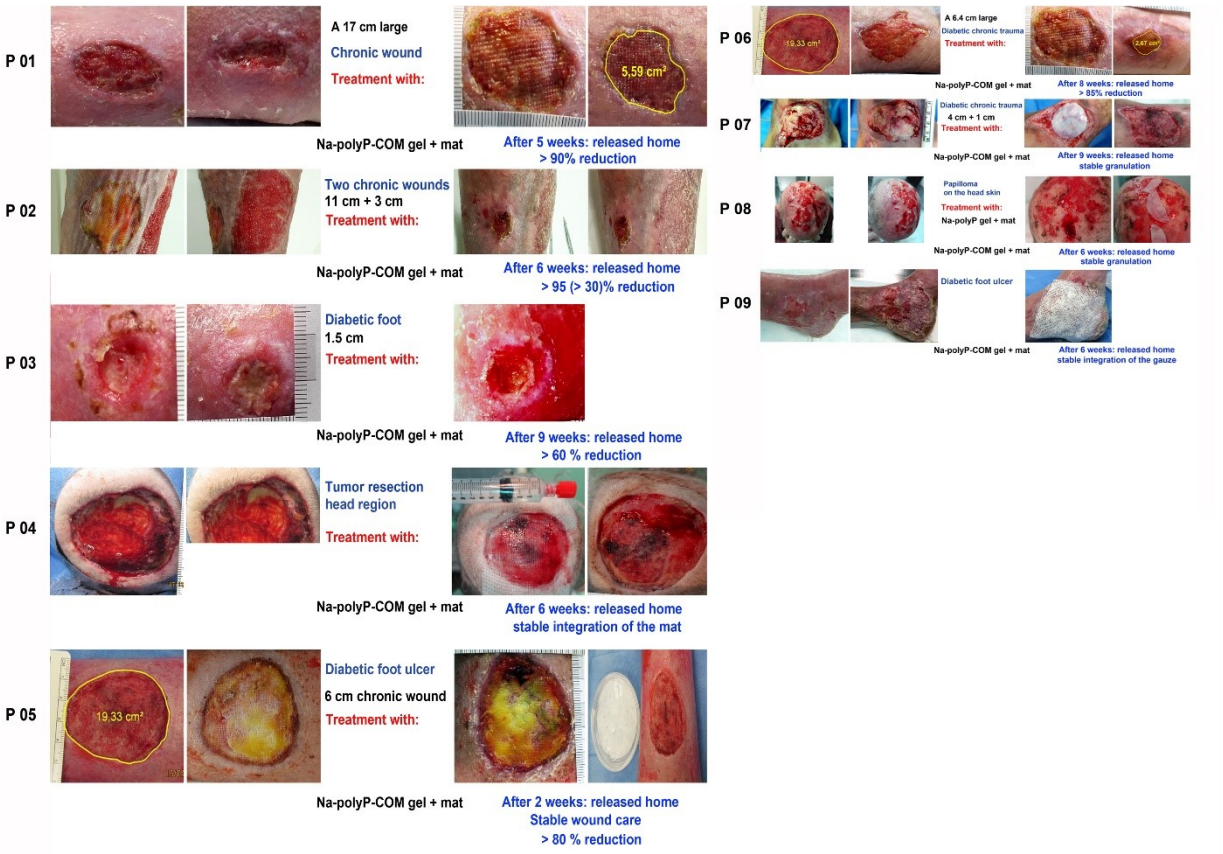

Fig. S-1. Complete series of Na-polyP-COM-treated patients in the proof-of-concept study. Previously described cases (images with permissions). All 9 patients (Patient P 01 to P 09) had been treated with Na-polyP-COM.

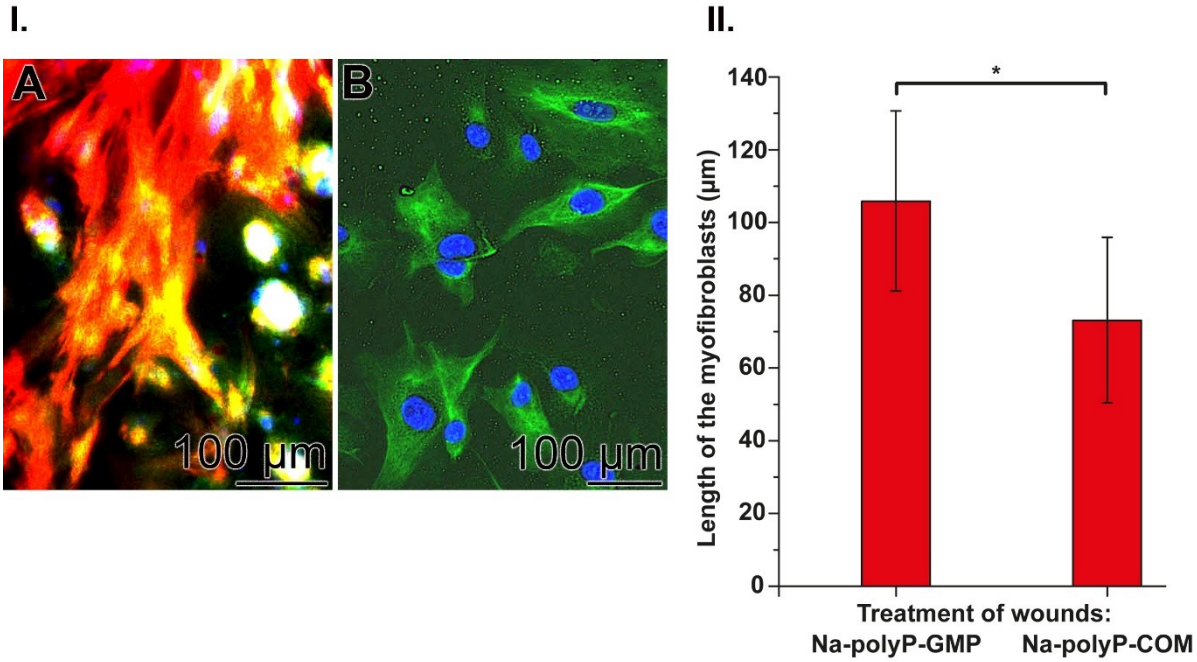

Fig. S-2. (**I.**) (**A**) During the exposure of the wounds to Na-polyP-GMP, the fibroblasts progress to the myofibroblast stage. Anti-human-α smooth muscle actin antibodies were used to identify the stress fibers in these cells. In (A), the immunocomplexes in the Na-polyP-GMP-treated wounds were visualized with anti-Alexa Fluor-350 secondary antibodies (in red). In wounds not treated with Na-polyP-GMP, the immunocomplexes were counterstained with a FITC-labeled secondary antibody (in green). In the latter case, the cytoplasmic actin structures in the fibroblasts (not the stress fibers) are also visualized by more intense coloration. (**II.**) Change in the length of the cells in the Na-polyP-GMP-treated wound; the chronic wound case documented here was chosen. The size of the fibroblasts in the Na-polyP-COM-treated wounds from the patients shown in Figure S-1 was determined. The correlation coefficient is significant ($p < 0.05$).

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