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Background

- ✓ Perioculars are delicate and sensitive cutaneous regions, which are highly susceptible to premature aging¹.
- ✓ Polynucleotide (PN) has shown clinical efficacy on skin rejuvenation². However, there is a gap in the literature supporting the use of PN in monotherapy treatments.
- ✓ **The aim of this study** was to perform *in vitro* characterization of a novel Swiss Polynucleotide regenerative biopolymer injectable complex, in terms of antioxidant and biostimulation activity.
- ✓ Additionally, we report the **clinical efficacy** for monotherapy periocular cutaneous rejuvenation.

Methods and results

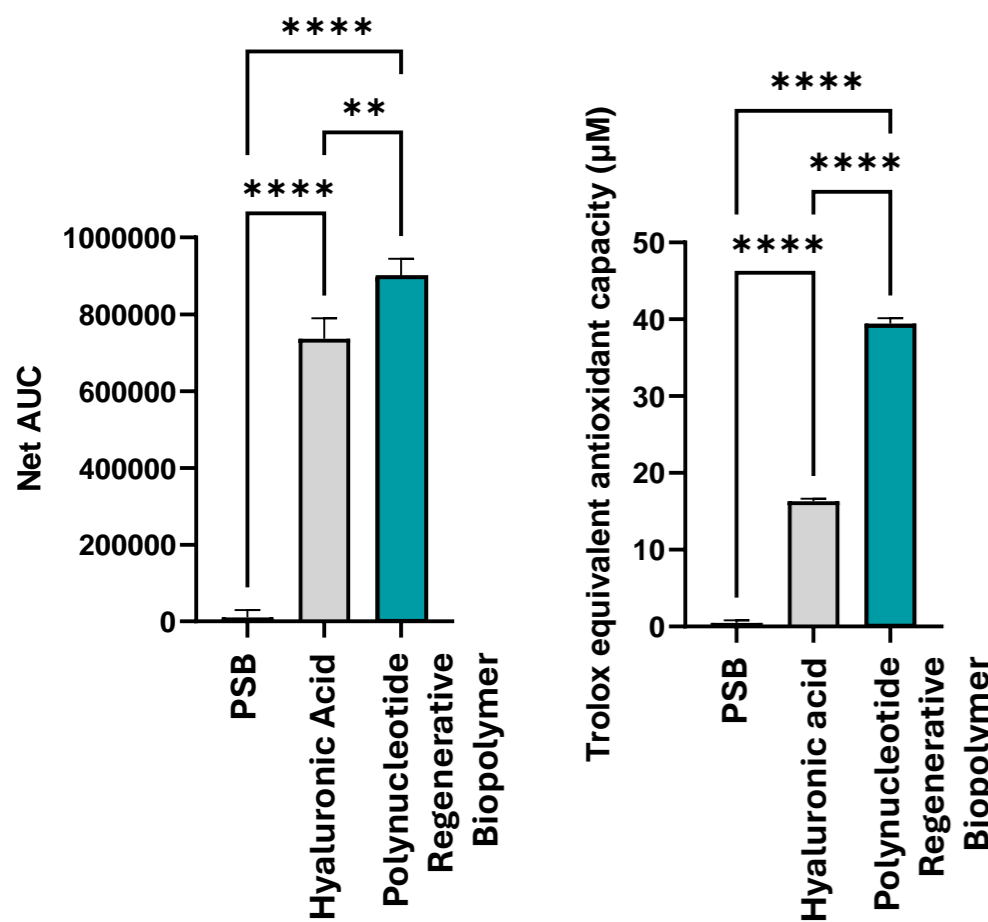
Polynucleotide regenerative biopolymer injectable complex showed higher antioxidant power than classical HA gel

Oxygen Radical Antioxidant Capacity (ORAC): The assay was conducted in a black, flat-bottom 96-well plate. Initially, 50 µL of fluorescein solution (8.4×10^{-8} mol/L) was added to each well to establish baseline measurements. Subsequently, 50 µL of either standards or sample solutions were introduced, followed by a 3-minute shaking period and a 15-minute incubation at 37°C. Immediately after the incubation, 50 µL of AAPH solution (153 mmol/L) was added to each well. Fluorescence was measured using a microplate reader with excitation and emission wavelengths set at 485/9 nm and 515/9 nm, respectively, and a reading height of 6 mm.

Cupric Reducing Antioxidant Capacity (CUPRAC): 50 µL of either standards or sample solutions were mixed with 150 µL of a reaction mixture, which consisted of equal volumes of Copper (II) chloride solution (10^{-2} mol/L), ammonium acetate buffer (pH 7), and ethanolic neocuproine solution (7.5×10^{-3} mol/L). The components were mixed in a 1:1:1 ratio. After an incubation period of 60 minutes, absorbance readings were taken using a microplate reader at a wavelength of 450 nm.

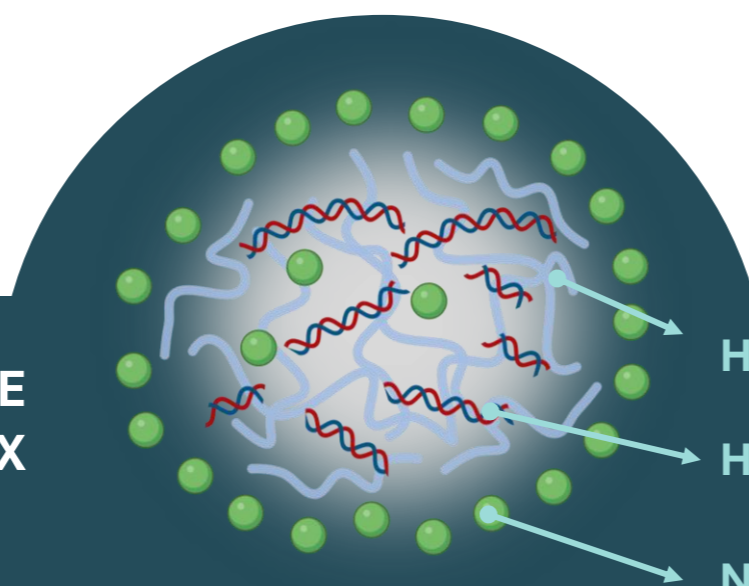
For both techniques: Measurements were performed in triplicate. Trolox standard solutions were used to generate the calibration curve for quantifying antioxidant capacity.

Innovyal Regenerative Action®: 12.5 mg/ml Polynucleotide Regenerative Biopolymer; Profhilo®: 32 mg/ml hyaluronic acid



Antioxidant action = double protection

- Anti-aging action**
Protects skin from free radical damage
- Formulation protection**
Protects formulation from natural degradation in the skin

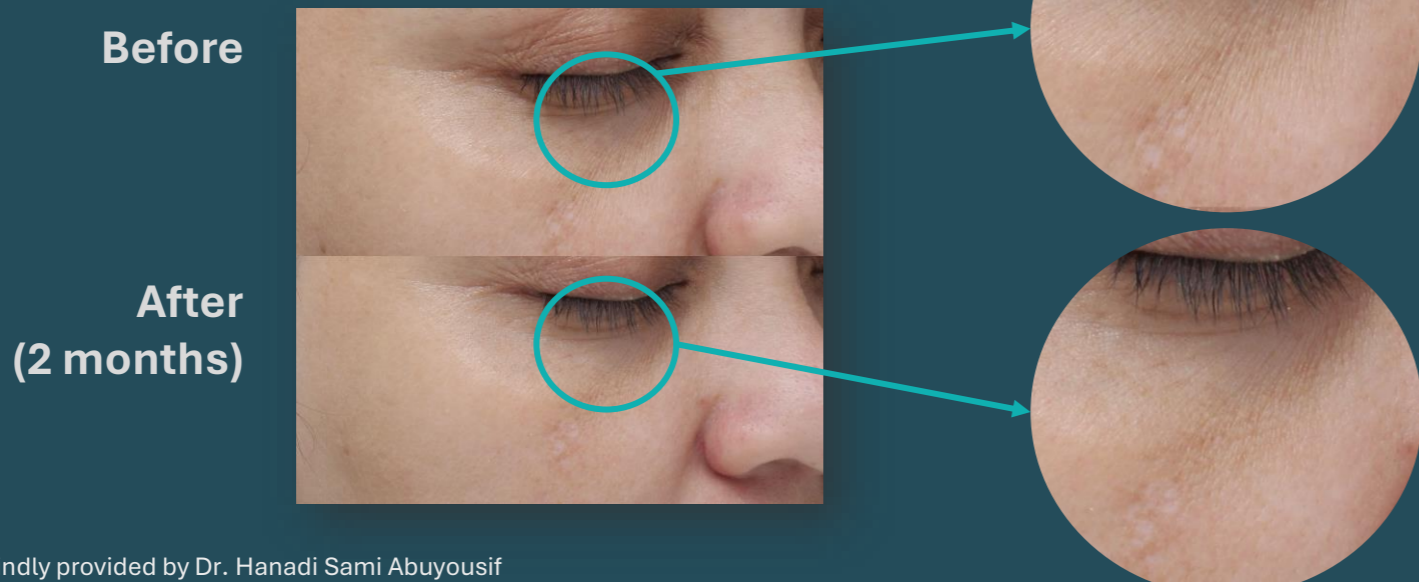


POLYNUCLEOTIDE REGENERATIVE BIOPOLYMERS COMPLEX

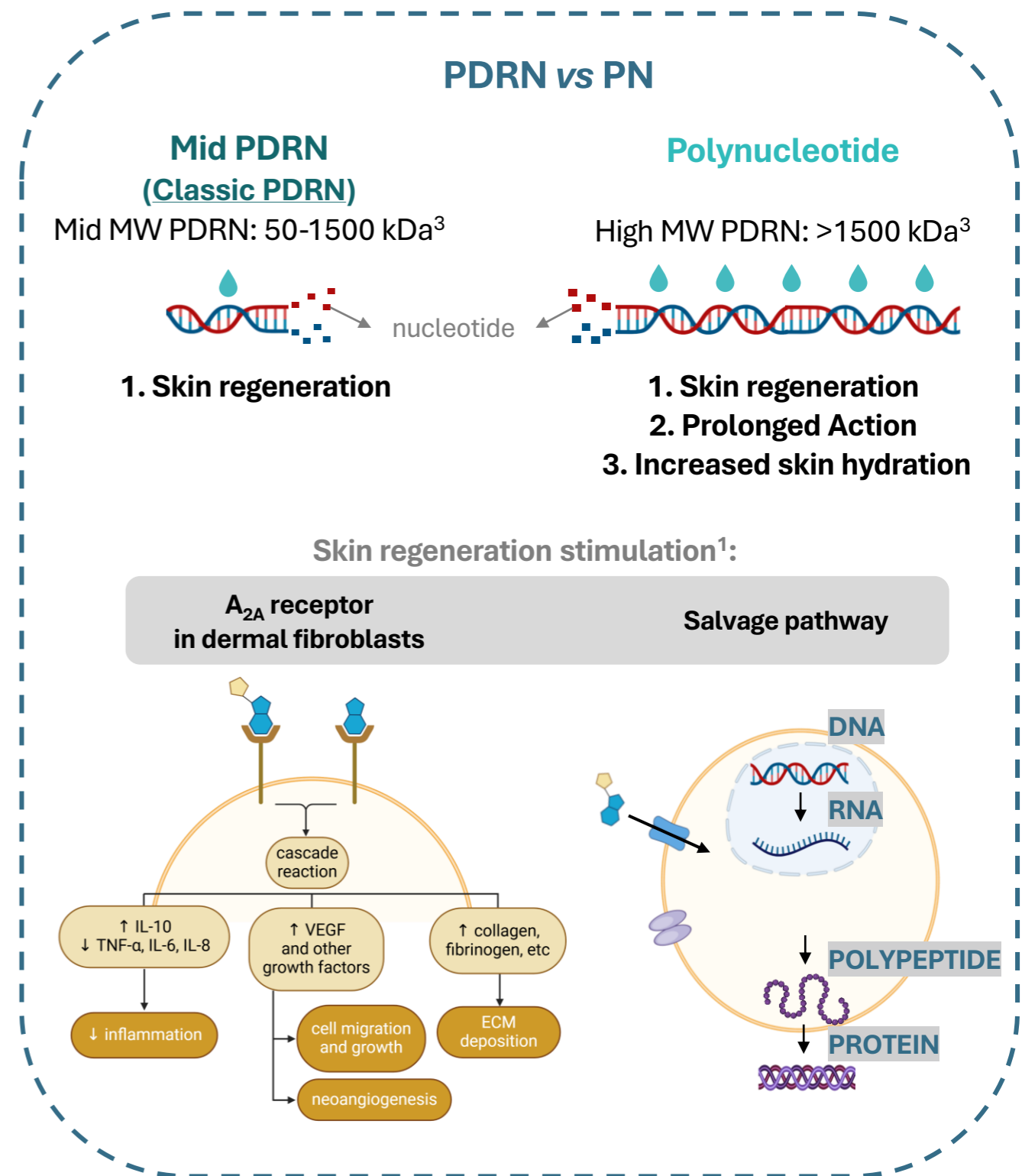
Clinical evidence

Polynucleotide regenerative biopolymer decreases fine lines after two administrations

Intradermal injection, administered with a 30 G needle using a multiple microalequate technique, involved applying 1.0 mL on each side of the face. Sessions were performed 14 days apart.

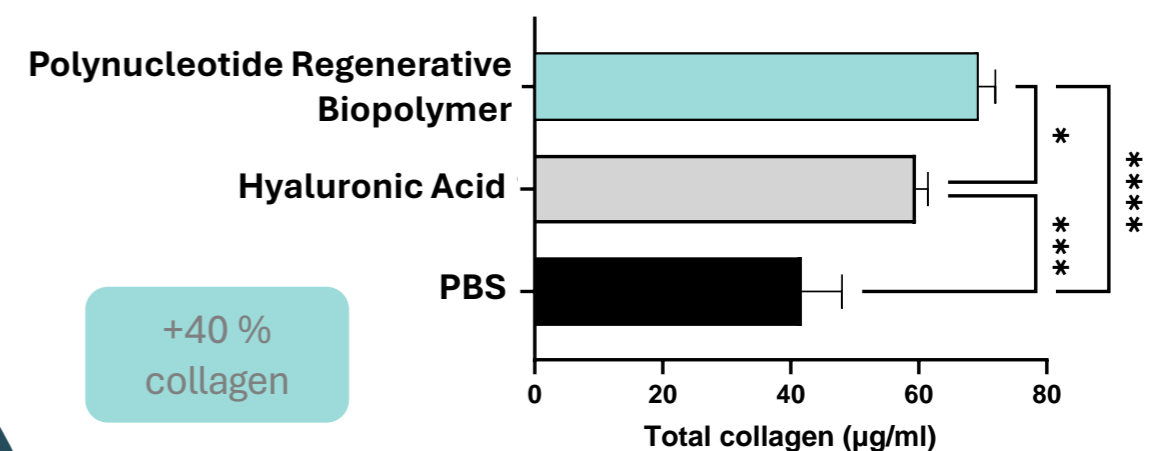


Images kindly provided by Dr. Hanadi Sami Abuyousif



Polynucleotide regenerative biopolymer injectable complex increases collagen production

To evaluate the neocollagenesis attributes, Innovyal Regenerative Action®, PROFHILO and PBS were incubated separately in contact with human primary fibroblast (DECH-2JM-Fib cell type) for 72 hours. After 72 h of incubation, the supernatant was removed and the wells were washed multiple times with PBS. The cells were then detached with TrypLE™ and hydrolyzed through cycles of freezing and thawing in order to measure the total collagen contents by fluorescence with the Collagen Assay Kit. In the first step of this procedure, collagen in the sample was enzymatically digested into peptides. Subsequently, the N-terminal glycine containing peptides reacted with the dye reagent to form a fluorescent complex. The fluorescence intensity of this product, which was measured at $\lambda_{ex} = 375/\lambda_{em} = 465$ nm on a Varioskan™ LUX was directly proportional to the collagen concentration in the sample.



Conclusions:

- The polynucleotide regenerative biopolymer has an effective antioxidant action
- The polynucleotide regenerative biopolymer effectively stimulates fibroblasts to produce collagen
- *In vitro* data is supported by clinical evidence that shows the reduction of fine line after treatment in monotherapy
- The study shows the effectiveness of the polynucleotide regenerative biopolymer, even in sensitive areas like periocular area

References:

1. Carruthers, A.; et al. A Validated Grading Scale for Crow's Feet. Dermatologic Surgery 2008
2. Cavallini, M.; et al. Consensus report on the use of PN-HPT™ (Polynucleotides Highly Purified Technology) in aesthetic medicine. J Cosmet Dermatol 2021
3. Hwang, K.-H.; et al. An Effective Range of Polydeoxyribonucleotides Is Critical for Wound Healing Quality. Molecular Medicine Reports 2018