Gold nanoparticles and near-infrared light as a new tool to enhance tissue regeneration

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Abstract Controlled temperature elevation within biological tissues, known as hyperthermia, holds promise as a therapeutic treatment. Its efficacy depends on several factors including timing, pulsing, and repetition. Recent research indicates the potential of heat-based therapies not only for cancer treatment but also in tissue regeneration. The usage of photothermal agents, such as gold nanoparticles, enables precise spatio-temporal heat generation, known as photothermal therapy (PTT). *Hydra vulgaris*, with their unique regenerative capabilities, serve as valuable models for exploring the effects of nanoparticles on tissue regeneration. AuNPs thanks to their plasmonic properties can induce physiological responses in the animals under near-infrared (NIR) irradiation, ranging from cell ablation to programmed cell death or thermotolerance. By tuning the NIR irradiation and the AuNPs dose, the capability of treated polyps to regenerate the missing heads under photostimulation will be dissected, at whole animal, cellular and molecular levels and compared to exposure to external macroscopic heat sources.

1 Introduction

Hyperthermia is a non-invasive technique that allows the controlled increase of temperature into biological tissues. Nowadays, there is an increasing attention to the use of gold nanoparticles to spatiotemporally generate heat, which is known as photothermal therapy (PTT). Gold nanoparticles are extremely biocompatible and can be finely tuned to absorb light in the near-infrared (NIR) spectral range (690-1100 nm) [1]. Beside the approaches based on thermoablation, i.e. anticancer strategies, the use of nanoparticles producing low amount of heat may be exploited to enhance tissue regeneration. Here, we propose *Hydra vulgaris* and its ability to regenerate missing parts to assess the potential of a particular class of gold nanoparticles to act as optical switchers and improve the tissue regeneration efficiency after an injury. Previous studies demonstrated the ability of gold nanoprisms (AuNPs) to induce thermo ablation [1,2,3]. To understand the impact of AuNPs on *Hydra* regeneration, we amputated the Hydra's head and monitored the regeneration for 72 hours. We compared the effect using a macroscopical external source (28°C) versus nanometric intracellular source (AuNPs). In particular, we analyzed four conditions: Untreated, Untreated NIR, AuNPs and AuNP-NIR. The regenerative process in *Hydra* is well known: 30-36 hours after amputation, small tentacle appears at the regenerating tip (stage 1). In the following 24 hours the tentacles elongate (stage 2), completing their growth in 72 h (stage 3). The graph in Fig.1A showed a significant increase of regeneration stages 2 and 3 in the animal treated with AuNPs and NIR irradiated. A similar result was observed in the animals exposed to 28 °C (Fig. 1B).

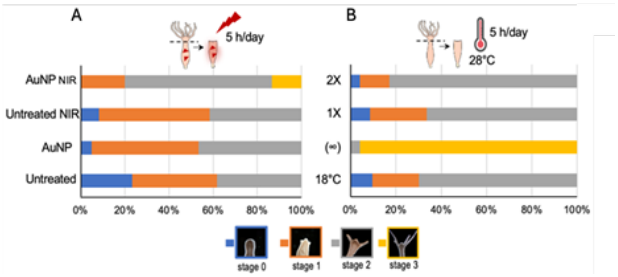

Fig 1. Regeneration assay. A) Polyps treated with AuNPs (1mg/ml), bisected and irradiated with NIR light. The percentage of regenerating heads has been scored every 24 h based on their developmental stage. B) Polyps bisected and exposed for different conditions to 28°C for 5 h /day. 1X = one pulse of heat (28°C), 24 h p.a.; 2X = two pulses of heat, 48 h p.a. Three independent biological replicates have been performed (n = 60).

To assess if the increased regeneration and reproduction in photostimulated polyps is correlated positively with the rate of cell proliferation, we performed a $BrdU^+$ assay.

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BrdU+ assay revealed a significant augmentation in stem cell proliferation after treatment with AuNPs coupled to NIR irradiation or exposure to 28 °C for 5 hours. To understand the involvement of molecular regulators of self-renewal and differentiation of *Hydra vulgaris*, we analyzed the transcription factors *forkhead box O* (FoxO) [4], *β-catenin* (*β-cat*) and the proto-oncogene *Myc1* engaged in Wnt/β-catenin signaling pathway and Superoxide dismutase gene (*SOD*) [5,1]. Five hours post AuNP-NIR irradiation, *β-catenin* and *Myc1* showed a significant downregulation, indicating their profound involvement in the regulation of interstitial stem cell (ISC) self-renewal. At the same time, *FoxO* and *SOD* showed upregulation, consistent with the recognized significant role of *FoxO* in self-renewal processes. Our results suggest that photostimulation of treated polyps induces improvement in ISCs. SOD generates a temperature increase through intracellular nanoheaters acting as a primary signal to trigger modulation of gene expression, as is observed even at 28 °C. According to these data, we hypothesized that the increase in regeneration efficiency is correlated with a temporal advance in the expression of genes involved in *Hydra* regeneration and development. Thus, we analyzed two genes: T-cell factor (*TCF*) and *Sp5* which activated in the initial phase of *Hydra*'s head regeneration [6,7]. The results showed that the improved regeneration may depend on a temporal acceleration of *TCF* and *SP5* expression in AuNPs irradiated animals, as occurs at 28°C.

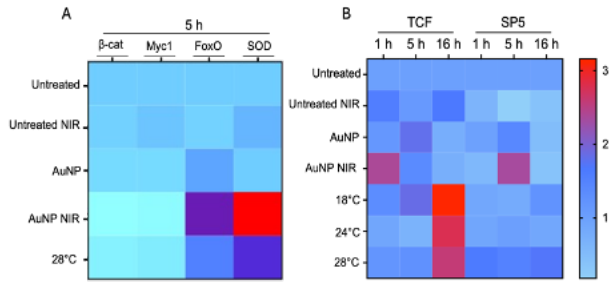

Fig 3. Heat-map quantitative assay. A) Gene expression levels by qRT-PCR modulated during regeneration, 5 h p.a. B) Time-course of *TCF* and *Sp5* during regeneration after AuNP-NIR treatment and at 28°C. Data are the average of three biological replicates, n=15, and three technical repeats.

2 Conclusion

The possibility to use nanometric intracellular heat source to enhance tissue regeneration represents an innovative approach with high impact in therapeutics. In our study, we demonstrated that AuNPs improved the Hydra regeneration capacity, increased the reproduction rate and stem cell self-renewal. Heat delivery externally or mediated by AuNP-NIR stimulations produced similar responses in modulating gene expression. Specifically, AuNP-NIR showed an upregulation of *FoxO*, *TCF*, *Sp5* and downregulation of *β-catenin* and *Myc1*, which may account for the accelerated regeneration observed *in vivo*. Our evidence reveals a unique function of nanoparticles that emit heat in controlling stem cell behavior through activation of molecular pathways, which could be targeted for regenerative medicine or wound healing treatment.

Acknowledgement

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