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Hairpin-structured probe conjugated nano-graphene oxide for the cellular detection of connective tissue growth factor mRNA

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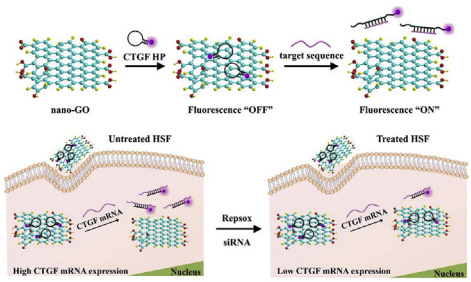
HIGHLIGHTS

- A fluorescent probe based on nano-graphene oxide and hairpin-structured probes (HPs) was constructed.
- This probe was employed to detect a potential abnormal scar biomarker CTGF mRNA in skin fibroblasts.
- This probe can achieve the successful identification of fibroblasts derived from hypertrophic scar.
- This probe also enabled the assessment of the effectiveness of anti-scarring drugs like Repsox and TGF-βRI siRNA.

GRAPHICAL ABSTRACT



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ABSTRACT

Identification of abnormal scars at their early stage has attracted increasing attentions as the scars can only be assessed qualitatively and subjectively upon maturity, when no invasive procedure is involved. This report introduces a fluorescent probe that targets a potential abnormal scar biomarker (connective tissue growth factor (CTGF) mRNA) in skin fibroblasts. This probe is constructed of hairpin-structured probes (HPs) targeting CTGF mRNA and the nano-graphene oxide (nano-GO) base. The HPs are non-covalently absorbed on the surface of nano-GO, which pre-quenches the fluorescence of HPs. Close proximity of complementary CTGF mRNA would lead to preferential HP hybridization and dissociation from nano-GO, which restores the fluorescence signal from HPs. Utilizing this probe, we can distinguish abnormal fibroblasts derived from abnormal scars and assess the effectiveness of anti-scarring drugs like Repsox and transforming growth factor-beta type I receptor (TGF-βRI) siRNA.

1. Introduction

Abnormal scars including hypertrophic scars and keloids exhibit distinct clinical characteristics in scar color, thickness, and pliability, which enables clinicians to assess and classify the scars through Vancouver Scar Scale [1]. However, this scar assessment is

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qualitative, subjective and resource-intensive, and only applicable for mature scars rather than newly-formed scars. Moreover, abnormal scars are traditionally treated by surgical excision when they are mature, which is associated with high recurrence rates [2]. Fortunately, early diagnosis of abnormal scars can be beneficial to scar prevention and treatment prior to excessive formation of fibrosis tissue [3]. Recent researches have reported that the population of fibrocytes (*i.e.* the precursors of fibroblasts) in abnormal scar tissue was higher than that in normal scar tissue, and thus may be an early index for distinguishing abnormal scars [4,5]. Nevertheless, identification of fibrocytes involved the invasive and time-consuming cryosectioning and immunostaining with expensive antibodies. To address this issue, potent strategies for non-invasively identifying the abnormal scars at their early stage are urgently needed.

mRNA has attracted growing interests as the biomarkers for the early disease diagnosis [6,7]. It could be examined through northern blotting [8] and polymerase chain reaction (PCR) [9] and probe-assisted fluorescence imaging [10]. The fluorescence imaging based on nucleic acid probes has been considered as a non-invasive approach for providing both temporal and spatial distribution of mRNA at the cellular level in a quantitative way [11,12]. Transforming growth factor-beta type I (TGF-βI) has been proven to be responsible for the fibrotic scarring [13]. Moreover, connective tissue growth factor (CTGF) mRNA, a TGF-βI downstream mediator, was shown to be overexpressed in fibroblasts from abnormal scars, and its down-regulation through TGF-βI inhibitor can significantly limit abnormal scarring progression [14–16]. This finding enables CTGF mRNA to be a potential biomarker for identifying abnormal scar fibroblasts. Recently, we have demonstrated that CTGF mRNA can be monitored with spherical-nucleic-acids in hypertrophic scar fibroblasts as well as in a pre-clinical rabbit ear wound model, which enabled the visual and spectroscopic quantification of abnormal fibroblasts in the dermis [17].

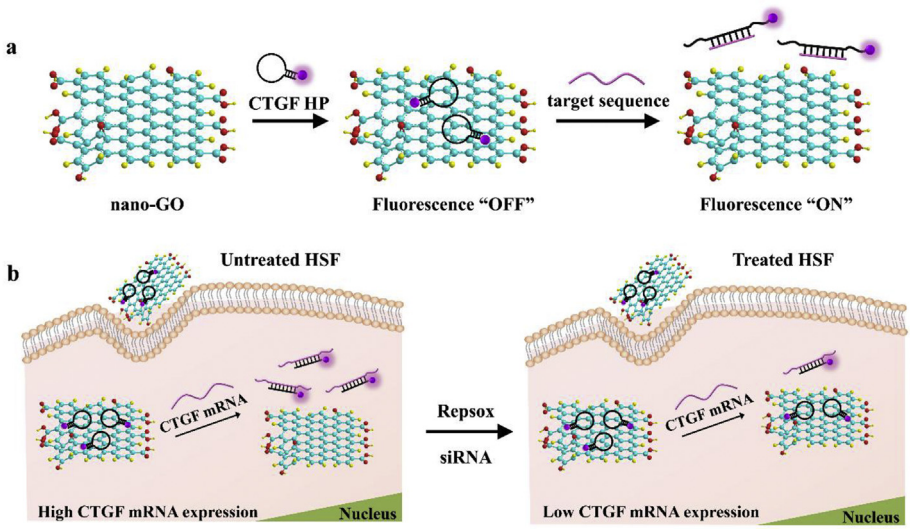
Nano-graphene oxide (nano-GO) nanosheets, a derivative of two-dimensional graphene, have received considerable attention as gene carriers for theranostics owing to their excellent properties including high water dispersibility, low cytotoxicity, easy functionalization and high fluorescence quenching capability [18]. nano-GO also shows high affinity to single-stranded oligonucleotide probes through the non-covalent interaction. Once inside a cell, nano-GO can protect the absorbed probes from nuclease unspecific digestion [19]. Moreover, the excellent fluorescence quenching ability of nano-GO would provide a low background and thus enable the sensitive response to analytes [20]. Although nano-GO has been applied for many applications such as apoptosis monitoring [21], cancer diagnosis [22], and induced pluripotent stem cell generation [23], it has not been adapted for skin cell identification.

This article introduces a nano-GO-based mRNA probe for monitoring CTGF mRNA expression in fibroblasts. This probe is constructed with a hairpin-structured probe (HP) targeting CTGF mRNA and nano-GO. The HPs are non-covalently absorbed on the surface of nano-GO due to the weak interaction between graphene aromatic plane and nucleobases. Nano-GO quenches the fluorescence of the dye on HP due to fluorescence resonance energy transfer (FRET) effect. The presence of the CTGF mRNA induces the formation of double-stranded oligonucleotides between HP and CTGF mRNA, which desorbs HPs from the nano-GO surface and restores the fluorescence of the dye on HPs (Scheme 1a). This probe can achieve the specific detection of the target sequence of CTGF mRNA as low as 0.7 nM. Through imaging the intracellular CTGF mRNA, it allows the identification of hypertrophic scars from normal fibroblasts. Furthermore, we can use it to assess the treatment effectiveness of the anti-scarring drugs like Repsox and specific siRNA through targeting transforming growth factor-beta type I receptor (TGF-βRI) (Scheme 1b).

2. Experimental

2.1. Reagents and materials

Graphite powder (99.8%, 325 mesh) was obtained from Alfa-Aesar. Repsox and all the oligonucleotides listed in Table S1 were purchased from Sigma-Aldrich (Singapore). Dulbecco's modified eagle medium (DMEM) with L-glutamine and 4.5 g/L D-glucose, fetal bovine serum (FBS), penicillin-streptomycin (10,000 U/mL), and trypsin-EDTA (0.5%) were obtained from Gibco Life Technologies (Singapore). Hoechst 33342 and Lipofectamine® 2000 were obtained from Thermo Fisher Scientific (Singapore). DNase I (1000 U/mL) and reverse transcription kit containing M-MLV RNase H (−) Mutant and dNTP were purchased from Promega (Singapore). iQ



Scheme 1. Schematic illustration of the working mechanism of the nano-GO/HP probe for CTGF mRNA detection. (a) Complexation and de-complexation of the probe; (b) Assessment of the effectiveness of anti-scarring drugs with the probe.

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