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Orally delivered thioketal nanoparticles loaded with TNF-α–siRNA target inflammation and inhibit gene expression in the intestines

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Small interfering RNAs (siRNAs) directed against proinflammatory cytokines have the potential to treat numerous diseases associated with intestinal inflammation[1]; however, the side-effects caused by the systemic depletion of cytokines[2–4] demands that the delivery of cytokine-targeted siRNAs be localized to diseased intestinal tissues. Although various delivery vehicles have been developed to orally deliver therapeutics to intestinal tissue[5–7], none of these strategies has demonstrated the ability to protect siRNA from the harsh environment of the gastrointestinal tract and target its delivery to inflamed intestinal tissue. Here, we present a delivery vehicle for siRNA, termed thioketal nanoparticles (TKNs), that can localize orally delivered siRNA to sites of intestinal inflammation, and thus inhibit gene expression in inflamed intestinal tissue. TKNs are formulated from a polymer, poly-(1,4-phenyleneacetone dimethylene thioketal), that degrades selectively in response to reactive oxygen species (ROS). Therefore, when delivered orally, TKNs release siRNA in response to the abnormally high levels of ROS specific to sites of intestinal inflammation[8–10]. Using a murine model of ulcerative colitis, we demonstrate that orally administered TKNs loaded with siRNA against the proinflammatory cytokine tumour necrosis factor-alpha (TNF-α) diminish TNF-α messenger RNA levels in the colon and protect mice from ulcerative colitis.

To localize the delivery of siRNA to diseased intestinal tissue, we identified the abnormally high levels of ROS produced at sites of intestinal inflammation as a disease-specific triggering mechanism for siRNA release[9]. For example, biopsies taken from patients suffering from ulcerative colitis[8,10,11], colon cancer[12] and *Helicobacter pylori* infections[9] have a 10- to 100-fold increase in mucosal ROS concentrations, which are confined to sites of disease development and correlate with disease progression. To orally deliver therapeutics to sites of intestinal inflammation we developed TKNs, which release encapsulated agents in response to ROS. The TKNs are formulated from poly-(1,4-phenyleneacetone dimethylene thioketal) (PPADT), a polymer composed of ROS-sensitive thioketal linkages[13] that are stable to acid-, base- and protease-catalysed degradation[13,14] (Fig. 1a). Therefore, orally delivered TNF-α–TKNs remain stable in the gastrointestinal tract, thereby protecting siRNA and preventing its release to non-inflamed tissues (Fig. 1b, upper panel). However, at sites of intestinal inflammation, the elevated ROS levels trigger the

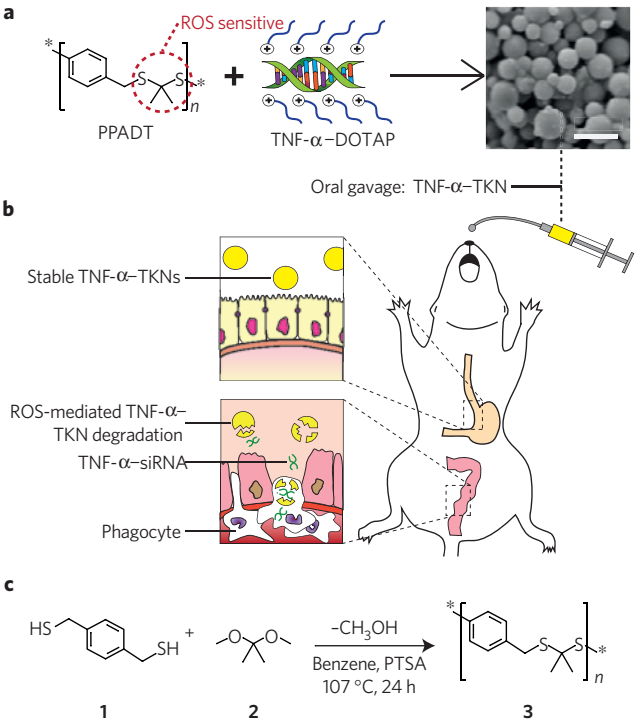

Figure 1 | Thioketal nanoparticles are formulated from a ROS-sensitive polymer and release orally delivered siRNA at sites of intestinal inflammation. a, PPADT **3** is a new polymer composed of ROS-sensitive thioketal linkages (circled in red). TNF-α–TKNs were prepared by first precomplexing TNF-α–siRNA with the cationic lipid DOTAP. Next, these TNF-α–DOTAP complexes were added to an organic solution containing PPADT. The scanning electron micrograph shows TNF-α–TKNs (scale bar represents 1.5 μm). **b**, When delivered orally, TNF-α–TKNs remain stable in the harsh environment of the gastrointestinal tract, protecting TNF-α–siRNA and preventing its release to non-inflamed mucosal tissues. However, at sites of intestinal inflammation, where infiltrating phagocytes produce unusually high levels of ROS, the TKNs degrade, thus releasing TNF-α–siRNA to the site of inflammation. **c**, PPADT **3** was synthesized using the acetal exchange reaction. PTSA: para-toluene sulphonic acid.

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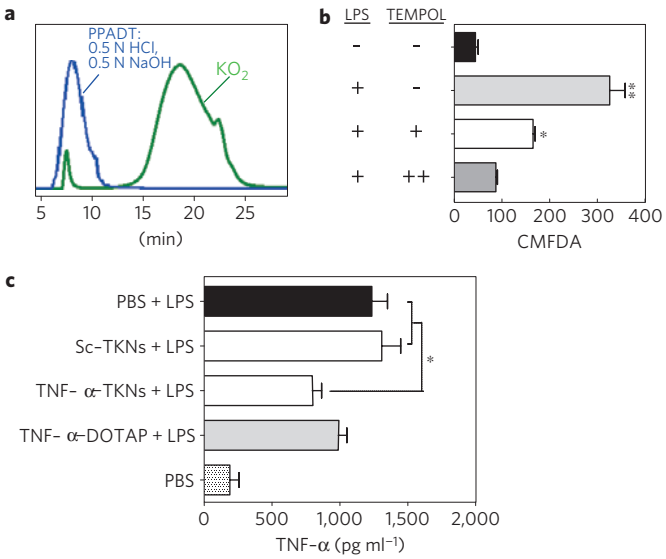

Figure 2 | PPADT is a ROS-sensitive polymer and nanoparticles formulated from it release their payloads in response to ROS produced by activated macrophages. a, GPC traces of PPADT before (blue) and after exposure to KO_2 (green). Incubating PPADT in acidic and basic environments (0.5 N HCl and 0.5 N NaOH) had no effect on the molecular weight of PPADT (coincident traces represented with blue line). **b**, Intracellular fluorescence of macrophages treated with CMFDA-loaded TKNs. The results are expressed as mean fluorescence ± standard error of the mean (s.e.m.) for $n = 3$ per group. Statistical significance was determined by a one-way analysis of variance (ANOVA) using Bonferroni's post hoc test ($^{*}p \leq 0.05$, $^{**}p \leq 0.001$). **c**, Extracellular TNF-α levels as determined by enzyme-linked immunosorbent assay. Macrophages treated with 23.0 μgTNF-α–siRNA per millilitre by means of TNF-α–TKNs and activated with LPS expressed significantly less TNF-α as compared with LPS-activated macrophages treated with PBS. The results are depicted as mean picograms of TNF-α per ml of media ± s.e.m. for $n = 3$ per treatment group.

degradation of the TNF-α–TKNs, thus localizing the release of siRNA to inflamed intestinal tissues (Fig. 1b, lower panel).

PPADT was synthesized from 1,4-benzenedimethanethiol **1** and 2,2-dimethoxypropane **2** using a step-growth polymerization that produces polymers with molecular weights (M_N) of approximately 9,000 Da (Fig. 1c). To investigate the specificity of PPADT for ROS, we incubated PPADT with either a superoxide solution, 0.5 N HCl solution or a 0.5 N NaOH solution and then analysed the resulting product's molecular weight using gel permeation chromatography (GPC). The GPC traces shown in Fig. 2a demonstrate that exposing PPADT to superoxide decreases its molecular weight from almost 9,000 Da to approximately 810 Da in 8 h, whereas incubating PPADT in either an acidic or basic environment had no effect on its molecular weight.

The unusually high concentrations of ROS localized to sites of intestinal inflammation are generated by activated phagocytes[15]. To determine the capability of the TKNs to release agents in response to a physiologically relevant source of ROS, we compared the amount of intracellular dye released from dye-loaded TKNs in activated (ROS overproducing) versus non-activated phagocytes. Macrophages were treated with TKNs loaded with 5-chloromethylfluorescein diacetate (CMFDA), washed of excess particles, and then activated with lipopolysaccharide (LPS). CMFDA is activated by intracellular proteases; therefore, the cellular fluorescence of cells treated with CMFDA-loaded TKNs (CMFDA–TKNs) will be proportional to the amount of CMFDA released from phagocytosed TKNs. Figure 2b shows that CMFDA–TKNs released CMFDA at an accelerated rate in response to ROS produced by activated macrophages. For example, LPS-activated macrophages showed a greater than sevenfold increase in cellular fluorescence compared with non-activated macrophages. This marked increase in cellular fluorescence was mitigated by treating LPS-activated macrophages with the ROS-scavenger 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPOL) (Fig. 2b), indicating that the amount of CMFDA released from CMFDA–TKNs is a function of ROS.

As a consequence of the essential role played by TNF-α in the onset and persistence of intestinal inflammation[16], we chose to treat mice suffering from dextran sodium sulphate (DSS)-induced colitis with TNF-α–siRNA (ref. 17). To formulate siRNA-loaded particles for *in vitro* and *in vivo* studies, we first complexed siRNA with the cationic lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), and then loaded these complexes into nanoparticles composed of PPADT. DOTAP has several properties that can increase the effectiveness of TNF-α–TKNs. Complexing siRNA with cationic species, such as DOTAP, enhances siRNA transfection by increasing siRNA stability[18], cellular internalization[19], mucosal transport[20] and endosomal escape[21,22]. Thus, at sites of intestinal inflammation, we anticipate that the co-encapsulated DOTAP will improve the efficacy of siRNA released in response to ROS in both the intracellular and extracellular environments. Furthermore, incorporating DOTAP endows nanoparticles with a positive surface charge (Supplementary Fig. S3), which can increase particle uptake by phagocytes[23] and adhesion to the negatively charged intestinal mucosa[24]. Finally, to optimize the particles for oral delivery, we engineered siRNA-loaded TKNs to have diameters of ~600 nm. Nanoparticles of this size limit nonspecific uptake by enterocytes[25], yet bind to inflamed colonic mucosa[26] and are efficiently taken up by phagocytes[21,23], which are the main producers of TNF-α at sites of intestinal inflammation[27].

To demonstrate the ability of TNF-α–TKNs to silence TNF-α expression by immune cells, we treated LPS-activated macrophages with TNF-α–TKNs or appropriate controls. Figure 2c shows that treating LPS-activated macrophages with TNF-α–TKNs resulted in a statistically significant reduction in TNF-α production as compared with cells treated with either PBS or TKNs loaded with a scrambled siRNA sequence (Sc–TKNs) ($p \leq 0.05$). In addition, we discovered that TNF-α–TKNs have a cytotoxicity profile that is similar to nanoparticles formulated from the FDA-approved material poly(lactic-*co*-glycolic acid) (PLGA) (Supplementary Fig. S4). These results show that TNF-α–TKNs can protect siRNA from serum, deliver TNF-α–siRNA in its active form and decrease the expression of TNF-α in activated phagocytes.

The stability of the TKNs to simulated gastrointestinal fluids (Supplementary Fig. S6) and their ability to release encapsulated agents, including siRNA (Supplementary Fig. S1), in response to ROS, motivated us to determine whether orally delivered TKNs could target siRNA to inflamed intestinal tissues. Intestinal inflammation was induced in female C57BL/6 mice by replacing their drinking water on day zero with a 3% solution of DSS (ref. 28). DSS supplementation induces an inflammatory response in the colon that mimics human ulcerative colitis and is characterized by: immune cell infiltration, a marked loss in body weight and elevated colonic ROS and cytokine production[27]. Starting on day zero, mice receiving either DSS or normal drinking water were given a daily oral gavage of TKNs loaded with a Cy3-tagged scrambled siRNA (Cy3siRNA). On day seven, the biodistribution of the delivered siRNA was measured by fluorescence. Our results demonstrate that the TKNs can localize orally delivered siRNA to sites of intestinal inflammation (Fig. 3a). For example, Fig. 3a shows a greater than threefold increase in the amount of Cy3siRNA delivered to the distal and proximal sections of the colons of mice receiving DSS and Cy3siRNA–TKNs as compared with

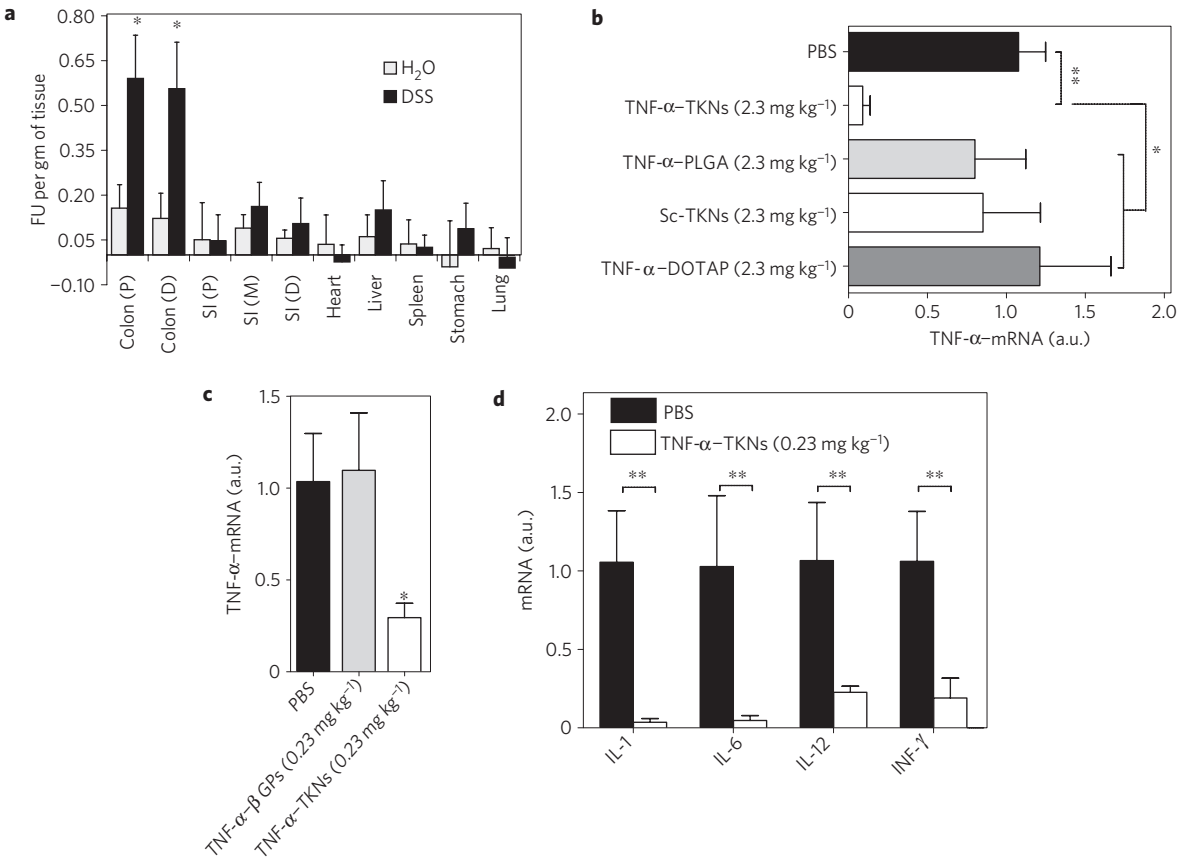

Figure 3 | TKNs target orally delivered siRNA to inflamed intestinal tissues and when loaded with TNF-α–siRNA reduce the colonic mRNA levels of proinflammatory cytokines in mice suffering from DSS-induced ulcerative colitis. a, Biodistribution of Cy3-tagged siRNA in the organs of mice treated with a daily gavage of Cy3siRNA–TKNs for 6 days (D: distal, M: medial, P: proximal, SI: small intestine). Fluorescent units (FU) per gram of tissue depicted as the mean ± s.d. for $n = 10$ mice per group. Mice received either a solution of DSS or water (H_2O). The asterisk represents statistical significance between the florescence in the same organs of DSS-treated mice and mice receiving water, and was determined using a two-sample Student *t*-test ($^*p \leq 0.05$). **b**, TNF-α mRNA levels in mice receiving DSS and treated with either scrambled- or TNF-α–siRNA (2.3 mg siRNA kg^{-1} d^{-1}) by means of either TNF-α–TKNs ($n = 6$), TNF-α–PLGA ($n = 5$), Sc-TKNs ($n = 5$) or TNF-α–DOTAP ($n = 6$). **c**, TNF-α mRNA levels in mice receiving DSS and treated with 0.23 mg TNF-α–siRNA kg^{-1} d^{-1} by means of either TNF-α–TKNs ($n = 10$) or TNF-α–βGPs ($n = 10$). **d**, Colonic cytokine mRNA levels in mice receiving DSS and treated with either PBS or 0.23 mg TNF-α–siRNA 0.23 kg^{-1} d^{-1} by means of TNF-α–TKNs ($n = 10$). Statistical differences in **a**–**d** were determined by a one-way ANOVA using Bonferroni's post hoc test ($^*p \leq 0.05$, $^{**}p \leq 0.001$).

mice receiving normal water and treated with Cy3siRNA–TKNs. Moreover, we observed relatively low levels of Cy3siRNA–TKNs delivered to the other organs of both treatment groups. As the inflammation induced by DSS is confined to the colon[28], these results confirm that the siRNA-loaded TKNs remain stable in non-inflamed regions of the gastrointestinal tract while targeting siRNA to inflamed intestinal tissues.

On the basis of these results, we hypothesized that orally administered TNF-α–TKNs could silence TNF-α expression in the colons of mice suffering from DSS-induced colitis. To test our hypothesis, mice receiving DSS were given TNF-α–siRNA or scrambled siRNA (2.3 mg siRNA kg^{-1}) encapsulated in TKNs by means of a daily gavage for five days. Starting on day zero, mice receiving DSS were also treated with free TNF-α–siRNA/DOTAP complexes (2.3 mg siRNA kg^{-1} d^{-1}) (TNF-α–DOTAP) or PBS. After seven days, the colonic mRNA levels of TNF-α and other proinflammatory cytokines upregulated as part of the intestinal inflammatory response, namely interleukin-6 (IL-6), IL-1 and interferon-gamma (IFN-γ), were analysed by real-time PCR. As shown in Fig. 3b, mice treated with TNF-α–TKNs (2.3 mg siRNA kg^{-1} d^{-1}) experienced a marked tenfold decrease in colonic TNF-α mRNA ($p \leq 0.001$). Analysis of colonic IL-6, IL-1 and IFN-γ mRNA levels showed that TNF-α–TKNs also inhibited the activation of these other proinflammatory signalling cascades (Supplementary Fig. S7).

To determine whether the ability of the TKNs to resist both hydrolytic- and enzymatic-mediated release (Supplementary Fig. S6) improves the efficacy of nanoparticle-mediated oral delivery of siRNA, we also treated mice receiving DSS with a daily gavage of TNF-α–siRNA (2.3 mg siRNA kg^{-1}) encapsulated in size- and charge-matched PLGA (50:50) nanoparticles (TNF-α–PLGA). Figure 3b shows that mice receiving DSS and treated with TNF-α–PLGA did not experience a significant decrease in colonic TNF-α mRNA, suggesting that the greater stability of the TKNs over that of PLGA nanoparticles to a simulated gastrointestinal environment (Supplementary Fig. S6) is an important factor in their improved efficacy. Although carrying out these experiments with TNF-α–siRNA-loaded nanoparticles formulated from a material similar to PPADT but without the thioketal linkages would provide further information about the efficacy of the TKNs, the synthetic methodology used to generate PPADT does not allow for the synthesis of such a material.

The high level of colonic TNF-α suppression achieved by the TNF-α–TKNs at a dose of 2.3 mg siRNA kg^{-1} d^{-1} motivated us to establish a minimum effective dose for TNF-α–TKNs. Starting on day zero, mice receiving DSS were treated with a tenfold

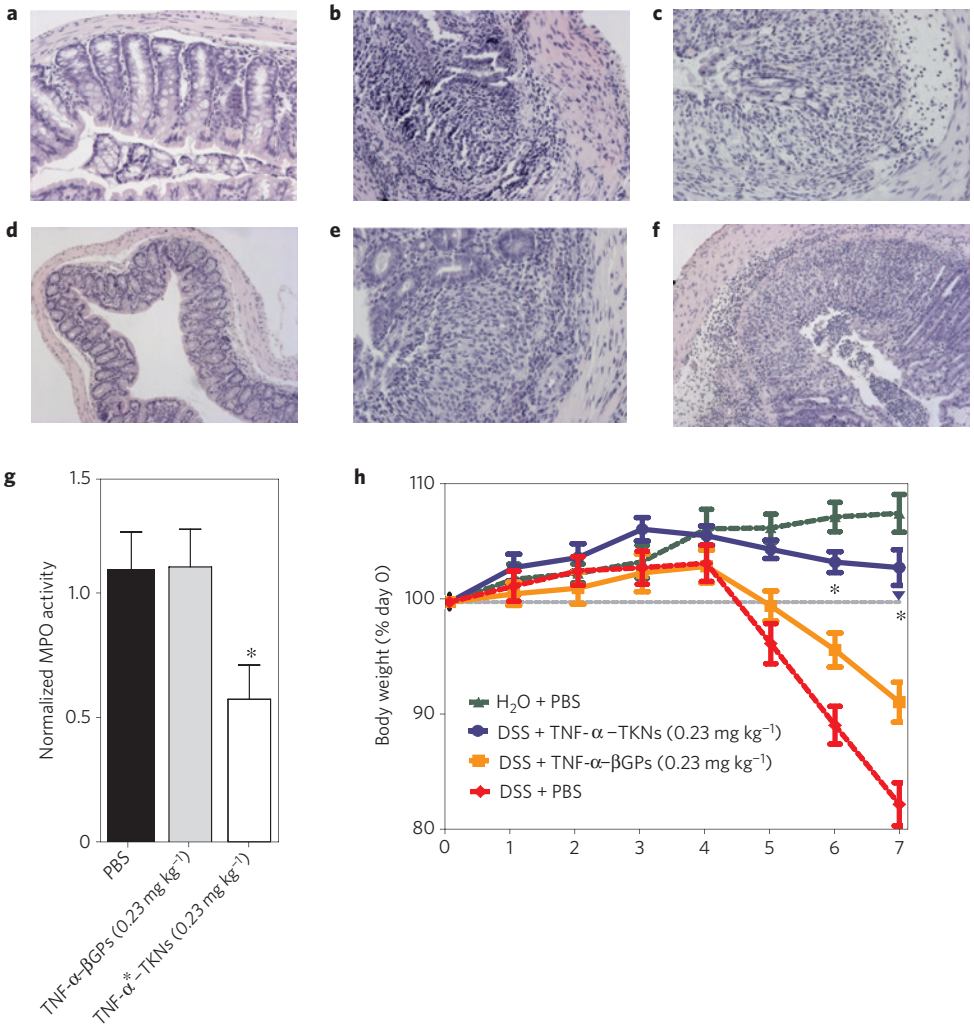

Figure 4 | Orally administered TNF-α–TKNs protect mice from DSS-induced colitis. a, Haematoxylin and eosin stained colon section from mice after seven days of receiving normal water and a daily oral gavage of PBS (×20 magnification). **b–f**, Haematoxylin and eosin stained colon sections (×20 magnification) from DSS-treated mice given a daily gavage of PBS (**b**), Sc-TKNs (2.3 mg kg^{-1}) (**c**), TNF-α-TKNs (0.23 mg kg^{-1}) (**d**), TNF-α-PLGA (2.3 mg kg^{-1}) (**e**) or TNF-α-βGPs (0.23 mg kg^{-1}) (**f**). **g**, Colonic MPO activity. The results are expressed as mUnits of MPO activity per milligram of protein and error bars represent ± s.e.m. Statistical significance was calculated using a one-way ANOVA and Bonferroni's post hoc test (*$p \leq 0.05$). **h**, Time course of mouse body weight. Mouse body weight was normalized as a percentage of day zero body weight. The body weight is depicted as the mean of each treatment group. The error bars represent ± s.e.m. The asterisk represents statistical significance from all other groups and was determined by a one-way ANOVA using Bonferroni's post hoc test (*$p \leq 0.05$).

lower dose of TNF-α–TKNs (0.23 mg TNF-α–siRNA kg^{-1} d^{-1}) by means of a daily oral gavage for five days. Figure 3c illustrates that at a tenfold lower dose TNF-α–TKNs continue to produce a significant inhibition of colonic TNF-α mRNA ($p \leq 0.05$) in mice receiving DSS. In addition, this approximately threefold decrease in TNF-α mRNA mitigated the activation of other proinflammatory signalling pathways that have been implicated in the development of ulcerative colitis, namely IL-6, IL-1 and IFN-γ (Fig. 3d). These results imply that orally delivered TKNs perform as well as present systemic delivery systems for siRNA that have been used to treat DSS-induced colitis[1].

To examine whether the ability of the TKNs to localize the delivery of siRNA to inflamed tissues plays a significant role in their ability to treat intestinal inflammation, we compared the efficacy of TNF-α–TKNs with TNF-α–siRNA-loaded β-glucan particles (TNF-α–βGPs), which have demonstrated the ability to orally deliver siRNA, but do not target diseased tissues[5]. Figure 3c shows that mice receiving DSS and treated with TNF-α–βGPs (0.23 mg siRNA kg^{-1} d^{-1}) did not experience a significant decrease in colonic TNF-α mRNA. These results imply that the ability of the TKNs to target inflamed tissues is also an important factor for their *in vivo* efficacy (see Supplementary Fig. S5 for characterization of TNF-α–βGPs).

Finally, we investigated whether orally delivered TNF-α–TKNs could allay the clinical manifestations of DSS-induced ulcerative colitis. Our results demonstrate that TNF-α–TKNs protected mice from DSS-induced colitis, as assessed by histological analysis, colonic myeloperoxidase (MPO)-activity and weight loss (Fig. 4, Supplementary Figs S9–S11). For example, the colons of mice receiving DSS and treated with TNF-α–siRNA (0.23 mg kg^{-1} d^{-1}) by means of TNF-α–TKNs had intact epitheliums, well-defined crypt structures and relatively low levels of neutrophil invasion (Fig. 4d). Furthermore, colonic MPO activity in mice receiving DSS and treated with TNF-α–TKNs (0.23 mg siRNA kg^{-1} d^{-1}) was markedly reduced (Fig. 4g). Finally, as depicted in Fig. 4h and Supplementary Fig. S11, mice treated with TNF-α–siRNA by means of TNF-α–TKNs were significantly heavier after seven days than mice receiving DSS and other treatments. In contrast, mice

receiving DSS and treated with TNF-α–PLGA, TNF-α–βGPs, Sc–TKNs, TNF-α–DOTAP and PBS showed all of the characteristics of DSS-induced inflammation as measured by histology, high levels of MPO activity and significant weight loss. These results demonstrate that among the strategies presented here the TKNs are uniquely suited to deliver siRNA and treat inflammatory diseases of the gastrointestinal tract (Fig. 4 and Supplementary Figs S7–S11).

Oral administration represents the most convenient and cost-effective means to deliver siRNA to diseased intestinal tissues. However, gastrointestinal fluids, the intestinal mucosa and cellular barriers to uptake represent significant obstacles for orally delivered siRNA. Here we present evidence that TKNs have the chemical and physical properties needed to overcome these obstacles and provide a therapeutic level of gene silencing in inflamed intestinal tissues. On the basis of our results, we expect that TKNs will make a significant contribution to the treatment of numerous gastrointestinal diseases linked to intestinal inflammation, including gastrointestinal cancers, inflammatory bowel diseases and viral infections.

Methods

PPADT synthesis and ROS sensitivity assay. Unless otherwise noted, all reagents were used as-received from Sigma. Briefly, a two-necked flask was charged with distilled benzene, 1,4-benzenedimethanethiol **1** (1.0 eq) and 2,2-dimethoxypropane **2** (1.0 eq.), then equipped with a metering funnel and distillation head for removal of the methanol by-product. The mixture was then stirred continuously and heated to 95 °C before a catalytic amount of re-crystallized *p*-toluenesulphonic acid (0.003 eq.) in distilled ethyl acetate was added to start the reaction. After 1 h, a solution of 2,2-dimethoxypropane and distilled benzene was added to the metering funnel and the funnel stopcock was set so that a small amount of 2,2-dimethoxypropane (0.08 eq per h) was add drop-wise over a period of 12 h. The reaction was allowed to stir overnight before the resulting polymer was isolated by precipitation in cold hexanes. The polymer was vacuum-dried and analysed by ^{1}H-NMR (Burker DMX 400, 400 MHz), ^{13}C-NMR and GPC (Shimadzu). ^{1}H-NMR: per repeating unit, (400 MHz, $CDCl_3$) δ ppm 7.28 (4H), 3.85 (4H), 1.60 (9H). ^{13}C-NMR: (400 MHz, $CDCl_3$) δ ppm 129.54, 77.56, 77.25, 76.93, 35.03, and 31.01. The molecular weight of the resulting polymer was approximately 9 kDa with a polydispersity of 1.8. To assess the ROS-sensitivity of PPADT, PPADT was exposed to superoxide according to the procedure described in ref. 19.

ROS-responsive release from TKNs *in vitro*. RAW 264.7 macrophages (American Type Culture Collection) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were seeded in 12-well culture plates (10^7 cells/well) and incubated with 0.2 mg ml^{-1} CMFDA–TKNs. After 3 h, the cells were washed with PBS three times, and then treated with either DMEM/FBS, DMEM/FBS spiked with LPS (5 μg ml^{-1}) or DMEM/FBS spiked with LPS and TEMPOL (2.5 mM and 5.0 mM). After 20 h, the medium was removed and the cells were washed with PBS and suspended in 1.5 ml of PBS containing 1% FBS and 5 mM EDTA. The cells were then passed through a 45 μm nylon mesh before being analysed by means of fluorescence-activated cell sorting (FACS) to assess intracellular dye release. FACS analysis was carried out on DB FACSVantageSE/DiVa instrumentation, and the results were analysed with FlowJow software.

Preparation of TNF-α–siRNA-loaded βGPs. βGPs containing TNF-α–siRNA were prepared exactly as described in ref. 12.

Induction of colitis and oral siRNA delivery. Animal experiments were carried out in female C57BL/6 mice (8 wk, 17–20 g, Jackson Laboratories). Colitis was induced by replacing their drinking water with a 3% (wt./vol) DSS (35,000 Da, (ICN Biochemicals)). For each of the animal experiments, groups of mice were treated with DSS or normal water for seven days. Mice were observed daily and evaluated for changes in body weight and development of the clinical symptoms of colitis. Starting on day zero, mice receiving DSS were given a daily gavage of PBS (200 μl) or a PBS solution (200 μl) containing one of the following: TNF-α–TKNs, TNF-α–PLGA, Sc–TKNs, TNF-α–βGPs or TNF-α–DOTAP. Mice receiving siRNA-loaded particles or TNF-α–DOTAP were treated with either 2.3 mg kg^{-1} or 0.23 mg kg^{-1} of TNF-α–siRNA or scrambled siRNA per day for six consecutive days (that is, days 0–5). Mice were killed on day seven, and histological assessment of colonic inflammation was carried out by haematoxylin and eosin staining of 5 μm colonic tissue sections, analysed by microscopy (×20 and ×10 magnification). All animal experiments were approved by The Animal Care Committee of Emory University, Atlanta (IACUC ID: 156–2008) and were carried out in accordance with the guide for the Care and Use of Laboratory Animals, published by the US Public Health Service.

***In vivo* targeting of Cy3-labelled siRNA to inflamed tissues using TKNs.** Mice receiving DSS or normal water were given a daily gavage of a PBS solution (200 μl) containing either empty TKNs or Cy3siRNA–TKNs (3.5 mg siRNA kg^{-1} d^{-1})(total of four groups with $n = 10$ per group). After seven days, each mouse receiving DSS had lost at least 10% of its body weight and had elevated fecal blood levels consistent with disease development. Organ samples (~0.6 mg) were removed, washed with cold PBS, patted dry with a paper towel and then homogenized in 1 ml of PBS (Polytron). Samples were then centrifuged at 20,000 r.p.m. for 30 min, and Cy3 was quantified in 100 μl of the supernatant using a fluorometer ($\lambda_{ex}/\lambda_{em} = 550/570$; Shimadzu). To correct for the background fluorescence resulting from the tissue and residual PPADT, the fluorescence measured from organ samples taken from mice receiving empty TKNs was subtracted from the fluorescence measured in samples taken from animals treated with Cy3siRNA–TKNs. The results are expressed as fluorescent units per gram of tissue.

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Author contributions

D.S.W. synthesized and characterized PPADT; formulated particles; designed, carried out and analysed experiments; and wrote the manuscript. G.D. designed, carried out and analysed experiments; and proof read the manuscript. L.W. carried out experiments. S.V.S. supervised the project. D.M. designed experiments; supervised the project; and proof read the manuscript. N.M. designed the synthetic strategy used to synthesize PPADT; supervised the project; and contributed to the writing of the manuscript.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/naturematerials. Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions. Correspondence and requests for materials should be addressed to D.M. or N.M.