Hydrogel Dopped with Nanoparticles for Local Sustained Release of siRNA in Breast Cancer

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Statement of Purpose: RNA interference (RNAi) has potential therapeutic application for a wide range of diseases (1,2). New targets for RNAi-based cancer therapy are constantly emerging. However, *in vivo* delivery of small interfering RNA (siRNA) remains a crucial challenge for therapeutic success because of degradation by RNAses. Moreover, targeting and retention of siRNA in normal cells is problematic due to risk of off-target or side effects. Here we present a new approach for siRNA delivery from biodegradable scaffolds with local retention of siRNA and for prolonged sustained release at the site of interest. The combination of adhesive hydrogels that specifically adhere to tumors, with degardable nanoparticles able to penetrate tumor cells while protecting and releaseing the siRNA cargo is a promising new approach in cancer therapy.

Methods: Biodegradable scaffolds were prepared by mixing varying ratios of poly(amido amine):dextran hydrogels and novel nanoparticles based on oligopeptide end-modified poly(β-aminoester)s (pBAEs) encapsulating siRNA. Release of fluorescently-labelled siRNA from different hydrogel-nanoparticle formulations was determined *in vitro* at 37ºC in PBS for seven days by fluorescence measurements.
The silencing efficiency of hydrogels dopped with siRNA-nanoparticles was assessed in MDA-MB-231 cells expressing green fluorescent protein (GFP) using anti-GFP siRNA.
In vivo gene silencing efficacy was determined in SCID mice mammary fat pad tumors derived from MDA-MB-231 cells expressing firefly luciferase (Luc). Scaffolds of 4mm diameter loaded with nanoparticles containing 10 μg of anti-Luc siRNA were implanted next to the mammary fat pad tumor (tumor volume=150 mm^3) and luciferase silencing was determined by bioluminescence measurements following intraperitoneal (IP) luciferin administration using the Xenogen IVIS device.

Results: A new family of oligopeptide-modified poly(β-aminoester)s with high transfection efficiency and low cytotoxicity was developed, when compared to previously described pBAEs and commercial transfection agents (3). Nanoparticles prepared with pBAEs and siRNA were successfully encapsulated in hydrogels prepared from oxidized dextran and poly(amido amine) (Fig. 1a). Release studies showed that siRNA was controlled released due to nanoparticle stabilization within the hydrogel and was determined both by hydrogel and nanoparticle composition (Fig. 1b), allowing fine tuning the release profiles. Moreover, *in vitro* experiments showed that siRNA silencing efficiency was maintained when nanoparticles were embedded in the hydrogel.

In vivo studies showed efficient luciferase silencing when siLuc-nanoparticles embedded inside the hydrogel were implanted in a murine breast cancer model expressing luciferase. In addition, siRNA release from hydrogel allowed silencing over a prolonged period of time and with enhanced efficiency when compared with a commercial reagent (Fig. 1c).

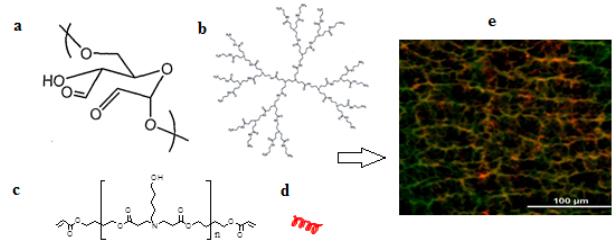

Figure 1a. (a) Oxidized Dextran (b) Poly(amido amine) dendrimer (c) PBAEs (d) siRNA forming a (e) hydrogel dopped with siRNA encapsulated in nanaoparticles.

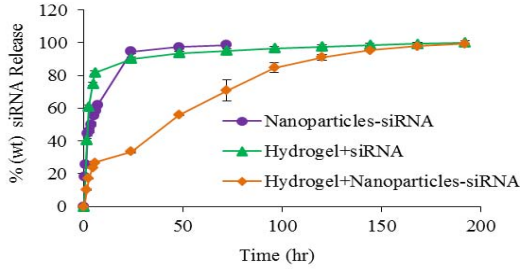

Figure 1b. siRNA release from nanoparticles, hydrogel and nanoparticles embedded in the hydrogel.

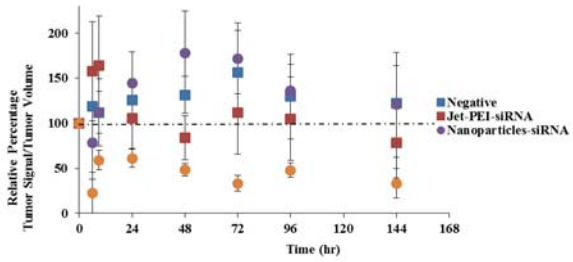

Figure 1c. Tumor state following anti-luciferase siRNA administration (no treatment, positive control, and hydrogel embedded nanoparticles).

Conclusions: A new platform for local siRNA delivery using biodegradable hydrogels and a new family of pBAEs nanoparticles for sustained release of siRNA is presented. The scaffold improves siRNA delivery efficiency with 2-fold increase in silencing compared to commercial reagent *in vivo*.

References:

1.Grimm, D., and Kay, M. A. (2007) *J. Clin.Invest.* (**117,** 3633–3641)
2. Behlke, M. A. (2006) *Mol. Ther.* (**13,** 644–670)