Nitric Oxide-Generating Poly(ethylene glycol) Copolymers for Prevention of Restenosis

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Introduction:

Procedures used to re-open occluded arteries, such as balloon angioplasty, often injure the arterial wall, removing the non-thrombogenic endothelial cells (EC) lining and triggering a cascade of events that can lead vessel re-narrowing, or restenosis. Platelet adhesion and aggregation at the injury site cause the release of mitogenic factors inducing smooth muscle cell (SMC) migration and proliferation, which can lead to the formation of an occlusive neointimal layer. Administering nitric oxide (NO) has long been proposed to aid in the prevention of neointimal thickening following balloon angioplasty, due to NO's ability to block a number of pathways in the restenosis cascade. NO's therapeutic benefits have not been realized, however, due to NO's short half-life *in vivo* and its many systemic effects.

Using these NO-generating polymers, we are able to deliver NO locally, thus achieving therapeutic levels of NO at the injury site without cytotoxicity. These NO-generating hydrogels been previously shown to inhibit both smooth muscle cell (SMC) proliferation and platelet adhesion, while increasing endothelial cell (EC) proliferation,[1] demonstrating their potential usefulness in the prevention of restenosis. Depending on material design, these materials can generate NO for up to 50 days and can be used to form thin coatings (~10 μm) on the luminal surface of an artery via interfacial photopolymerization[2] to provide local and sustained NO therapy following vascular injury. This study evaluated the ability of these NO-generating hydrogels to inhibit intimal thickening, a major component of restenosis, in a rat carotid injury model.

Experimental:

NO-Releasing PEG hydrogels:

PEG-NO-nucleophile was formed by reacting polyethylene glycol N-hydroxysuccinimide monoacrylate (ACRL-PEG-NHS; MW 3.4 kDa) with cysteine (Cys). The resultant copolymer was dissolved in water and reacted with an equimolar amount of sodium nitrate at pH 2 to form the hydrogel precursor, PEG-Cys-NO. The pH was the adjusted to 7.4 and the PEG-Cys-NO was incorporated into the PEG hydrogel precursor (MW 6000 g/mol), which contained 20 % (w/v) PEG-diacrylate and 1.5 % (v/v) triethanolamine in HBS, 3.95 % (v/v) *N*-vinylpyrrolidone, and eosin Y as a visible light photoinitiator. NO-releasing materials were incubated at 37˚ C in HEPES buffer and assayed for NO release using the Griess assay.

Rat carotid balloon injury model:

Polymer precursors were prepared as described above and sterilized by filtration. The left carotid artery of Sprague-Dawley rats (425-450 g, anesthetized with isoflurane) was denuded three times with a 2F Fogarty catheter. Immediately following the injury, either a NO-generating PEG-Cys-NO hydrogel precursor solution (1.25 μmol NO delivered) or control PEG-diacrylate hydrogel precursor solution was applied perivascularly and photocrosslinked *in situ*. The carotid arteries were harvested after 4d and 14 d for histological evaluation.

Results:

PEG-Cys-NO hydrogels produced nitric oxide over a period of approximately 2 hr at pH 7.4, with most of the release occurring within the first 30 min. The perivascular application of our NO-generating polymers post-injury reduced neointimal formation by approximately 80%

compared to controls (intimal area/medial area: PEG-Cys-NO = 0.20 ± 0.17, control = 0.84 ± 0.19, $p < 0.00002$; intimal thickness: PEG-Cys-NO = 12.7 ± 10.4 µm, control = 60.4 ± 18.0 µm, $p< 0.00002$).

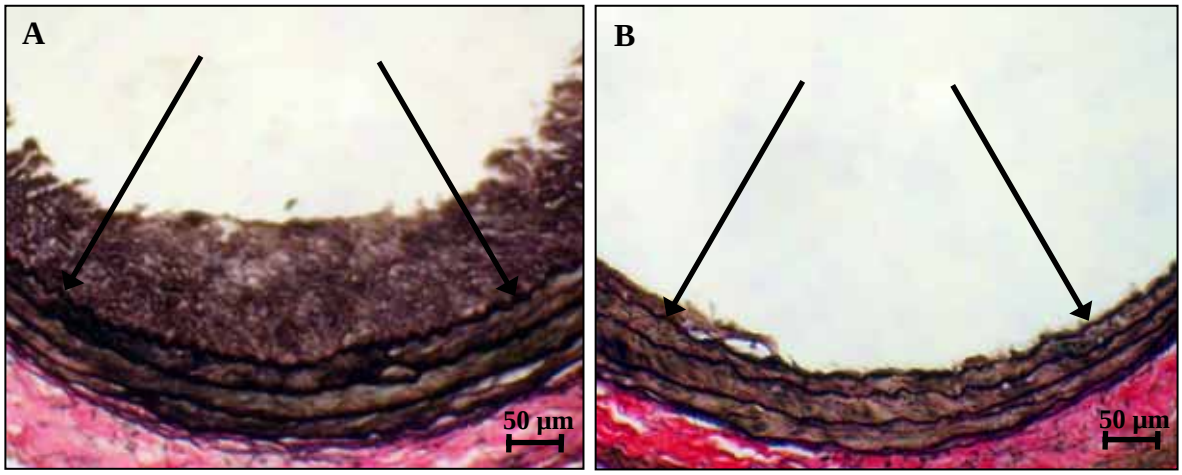

Figure 1: Neointimal formation was significantly inhibited by treatment with PEG-Cys-NO hydrogels. Histological sections of injured arteries treated with (A) PEG-DA control hydrogel and (B) PEG-Cys-NO hydrogel. Arrows indicate the internal elastic lamina.

To examine cell proliferation, histological sections were immunostained for proliferating cell nuclear antigen (PCNA). Treatment with the PEG-Cys-NO hydrogels caused a significant decrease in the percent of medial cells that stained positive for PCNA (28.9 ± 4.7 %) as compared to treatment with the PEG-DA control hydrogels (51.3 ± 0.6 %, $p<0.02$). In uninjured right vessels, 4.7± 1.0% of cells in the medial layer stained positive for PCNA. Additionally, vessel re-endothelialization appeared to be slightly enhanced in the presence of the NO-generating hydrogels. Application of the control hydrogel did not result in any difference when compared to untreated arteries.

Discussion:

The current study has described the synthesis and characterization of a new group of biomaterials that can be photopolymerized *in situ* to coat tissues and that produce NO for prolonged periods of time. These data indicate that localized delivery of NO from these hydrogels can significantly inhibit neointimal formation in a rat carotid balloon injury model. The combined effects of these NO-generating hydrogels make them well suited for use as a stent coating or in endoluminal paving to prevent restenosis.

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References:

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2. Hill-West, J. L., S. M. Chowdhury, et al. (1994). Proc Natl Acad Sci U S A 91(13): 5967-71.