Antioxidant citric-acid based polymers inhibit intimal hyperplasia

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Statement of Purpose: Restenosis as a result of intimal hyperplasia (IH) remains a significant problem for vascular interventions. This is thought to occur due to excessive inflammatory response, oxidative stress, and the loss of NO bioavailability. In particular, reactive oxygen species (ROS) play an important role in NO bioavailability as excessive amounts scavenge NO. Furthermore, free iron is also known to raise levels of ROS. Therefore, vascular devices that use biomaterials with the capability to reduce the effects of oxidative stress through anti-oxidant and metal chelating properties may potentially be beneficial to reduce the deleterious effects of ROS on IH. Herein we describe polymers with intrinsic and sustained antioxidant and metal chelating properties.

Methods: Poly(octanediol-co-citrate) (POC) and poly(1,8 octanediol-co-citrate-co-ascorbate) (POCA) were synthesized. POC prepolymer was made by mixing 1,8-octanediol and citric acid in 1:1 ratio and heating at 160°C for 10 minutes, followed by 140°C for an additional 60 minutes. For POCA, ascorbic acid was added at 10% molar ratio. Prepolymers were post-polymerized for 4 days at 80°C. Polymer formation was confirmed using FTIR and NMR analysis. Antioxidant activity was measured using the 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assay to assess free radical scavenging ability and β-carotene assay to assess inhibition of lipid peroxidation. Iron chelation was measured using a ferrozine competition assay. To assess reduction of cellular ROS generation *in vitro*, human umbilical vein endothelial cells (HUVECs) were incubated with polymers for 2 hours, with dichlorodihydrofluorescein (DCF, 10 uM) and subsequently challenged with hydrogen peroxide. Fluorescence development was measured at Ex/Em 485/535 nm. HUVEC and human aortic smooth muscle cell (HASMC) proliferation on the polymers was assessed by quantifying DNA content over time. In vivo IH levels were evaluated after 4 weeks in a guinea pig aortic interposition model with POC- and POCA-coated ePTFE.

Results: HUVECs showed increased proliferation on POC and POCA compared to tissue culture plastic, while HASMCs had decreased proliferation (data not shown). POC and POCA both had significant antioxidant effects, scavenging free radicals and inhibiting lipid peroxidation, with POCA having a greater effect (**Figure 1**). The antioxidant effect of both polymers might be explained by the resemblance of the polymer network to triglycerides, which are known to have antioxidant properties. Both POC and POCA exhibited strong iron chelating properties, showing retention of metal chelation capability by the citric acid. ROS generation in HUVECs was also diminished when the cells were exposed to both polydiolcitrates, while IH levels were reduced in both POC- and POCA-coated ePTFE grafts in vivo (**Figure 2**).

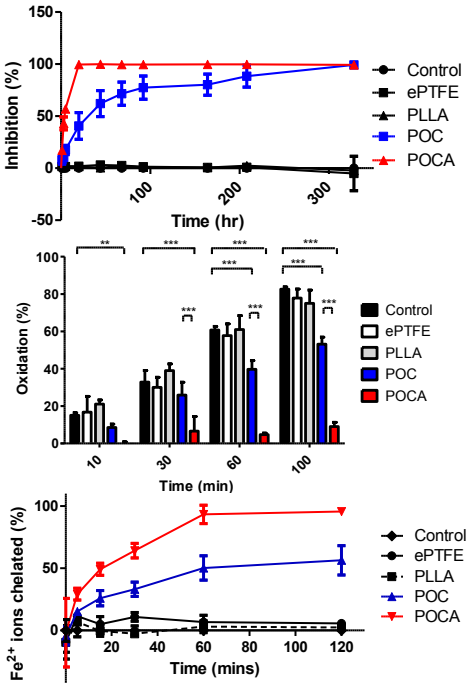

Figure 1. Top: ABTS assay shows free radical inhibition by both POC and POCA, whereas PLLA and ePTFE have no such activity. Middle: POC and POCA inhibit lipid peroxidation. Bottom: POC and POCA both chelate iron ions.

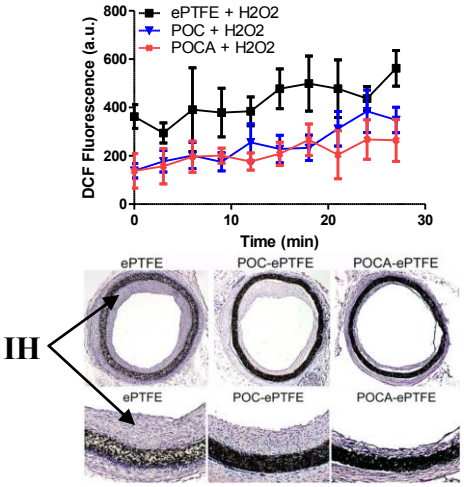

Figure 2. Top: HUVECs exposed to POC and POCA were resistant to ROS generation after H_2O_2 challenge. Bottom: POC- and POCA-coated ePTFE grafts inhibit intimal hyperplasia in a guinea pig aortic interposition model

Conclusions: POC and POCA show intrinsic antioxidant and iron chelating properties, which may be protective to cells that are in an environment of excessive ROS generation. POC and POCA also show a decrease of smooth muscle cell proliferation and reduction of IH levels in vivo. The use of polydiolcitrates in vascular devices such as grafts and stents may therefore improve redox status and reduce intimal hyperplasia after vascular intervention.