Oligo(trimethylene carbonate) For Vascular Endothelial Growth Factor Delivery

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Statement of Purpose: Localized growth factor delivery is a potentially useful approach for therapeutic angiogenesis treatment of ischemia. One challenge to overcome to make this goal a reality is the development of a suitable delivery vehicle. The delivery vehicle must meet a number of criteria: be easily implanted, provide multi-week delivery, be biocompatible and preferably biodegradable, and maintain the bioactivity of the growth factor during delivery. It was the objective of this study to explore the potential of oligo(trimethylene carbonate) as such a delivery vehicle. Poly(trimethylene carbonate) is a biodegradable, biocompatible polymer with a low glass transition temperature. It was reasoned that preparation of a trimethylene carbonate oligomer would result in biodegradable liquid polymer that could be readily loaded with solid vascular endothelial growth factor (VEGF) particles and injected into an ischemic tissue site. Therefore, various molecular weight oligomers were synthesized and their thermal properties and melt viscosity measured. VEGF co-lyophilized with trehalose was loaded into selected oligomers and released *in vitro*. The biodegradation rate and biocompatibility of the oligomers was also assessed *in vivo*.

Methods: Linear poly(trimethylene carbonate) of varying molecular weight was synthesized by ring-opening polymerization at 120ºC for 24 hours, catalyzed by zinc ethylhexanoate and initiated by octanol in vacuum-sealed glass ampules. The number average molecular weight (M_n) of the oligomers was measured by ^{1}H NMR, and the viscosity at various temperatures ranging from 34ºC to 40ºC using a Reologica ViscoTech controlled stress rheometer. 10 mg of trehalose, 0.5 mg of serum albumin and 0.01 mg of VEGF were co-lyophilized in 0.5 mM pH 5 succinate buffer. The resulting powder was ground and sieved to yield < 50 μm particles. These particles were stirred into pre-warmed (35 ºC) oligomers using a mortar and pestle. The loading of lyophilized particles was 1 w/w%. The suspension was drawn into a plastic 1 ml syringe, and then 0.5 ml dispensed into the base of a 2 ml glass vial. To the vials was added 1 ml of 37ºC pH 7.4 PBS containing 0.02% polysorbate 20 and 0.02% sodium azide. The vials were stirred and maintained at 37ºC. At frequent sampling intervals, the buffer was replaced with fresh buffer. Growth factor concentration in the release medium was measured by ELISA (Peprotech). The bioactivity of the released VEGF was assessed by determination of its ability to stimulate the proliferation of human aortic endothelial cells. *In vivo* biocompatibility and biodegradation of the oligomers alone were assessed by subcutaneous and intramuscular injections of 100 mg into 2 sites in male Wistar rats.

Results/Discussion: Oligo(trimethylene carbonate) is an amorphous, viscous, hydrophobic liquid at room temperature. Its melt viscosity at 37ºC ranged from 2 to 79 Pa·s as its M_n increases from 900 to 2300 g/mol. The VEGF particles were easily distributed throughout the polymer by physical mixing, and the resulting dispersion, pre-warmed to 37ºC, could be injected through a 18.5 gage needle, provided the molecular weight was less than about 2000 g/mol. *In vitro* release of the VEGF was continuous, with a release rate of approximately 5 ng/day (Fig 1). The released VEGF was bioactive, as assessed via the human aortic cell proliferation assay. The implanted oligomers were well tolerated by the animals. The rats all gained weight and showed no signs of discomfort. For example, after 4 weeks, 86 w/w% of the 1500 g/mol subcutaneously implanted oligomer had been degraded. ^{1}H NMR analysis showed that there was only oligo(trimethylene carbonate) in the retrieved oligomer, and that its molecular weight after 4 weeks had increased from initially 1550 g/mol to 2300 g/mol. These results indicate that degadation is rapid and that the low molecular weight fraction of the oligomer had degraded preferentially. Histological analysis of the tissue showed only a very thin fibrous encapsulation layer around the subcutaneous implants and no fibrous encapsulation of the intramuscular implants. The intramuscularly implanted polymer had become dispersed as droplets in the muscle tissue due to muscle action.

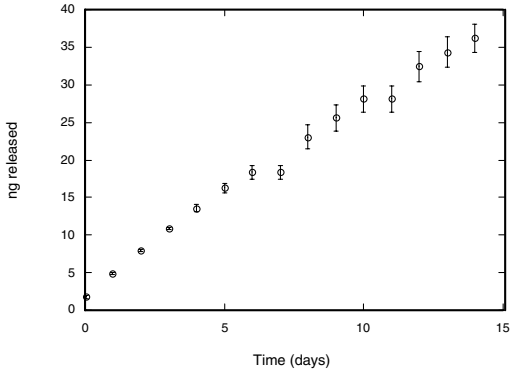

Figure 1. Release of VEGF from 1550 g/mol oligo(trimethylene carbonate) into PBS at 37 ºC. Each point represents the average and the error bars represent the standard deviation of release from 3 samples. Approximately 50 ng of VEGF was incorporated.

Conclusions: Oligo(trimethylene carbonate) has many suitable qualities to serve as an effective injectable depot for local VEGF delivery. Its viscosity is readily adjustable, it can be injected through a standard gage needle, the VEGF maintained high bioactivity *in vitro*, the oligomer is well-tolerated and it degrades rapidly *in vivo*. Future work should include an assessment of the efficacy of the released VEGF in stimulating angiogenesis, and a more complete examination of the host reaction to the implanted polymer.