Bioactivity of TGF-β1 and PDGF-BB Released from PEGylated Fibrin Gels

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Statement of Purpose:

Transforming growth factor-β1 (TGF) has demonstrated ability to upregulate cell markers of the mesenchyme from bone marrow progenitor cells,[1] while the BB isoform of platelet-derived growth factor (PDGF) can induce migration and regulation of pericyte progenitor cells.[2] PDGF has a short time course in comparison to TGF and thus early release is optimal while sustained release for TGF is appropriate. The aim of the current study is to sequester TGF within a fibrin gel either via a bifunctional succinimidyl α-methylbutanote poly(ethylene glycol) (PEG-$(SMB)_2$) linkage or direct affinity binding with the matrix. Simultaneously, PDGF will be entrapped within fibrin gels due to the relatively low affinity of PDGF for fibrinogen (Fib). PEGylating the fibrin gel alters the degradation rate and the differing binding schemes allows for temporal control of growth factor (GF) release.

Methods:

Porcine Fib (Sigma) was PEGylated with PEG-$(SMB)_2$, 3400 MW (Nektar Therapeutics) in a 1:10 molar ratio. The reaction occurred over 1 hour at 37ºC in TBS, pH 7.8. Human TGF (R&D Systems) was immediately added to the PEGylated Fib, and reacted at 37ºC for 30 minutes. Dialysis with a 100,000 MWCO membrane against TBS, pH 7.8 was performed overnight. Human PDGF (R&D Systems) was added to the PEGylated Fib solution and reacted for 30 minutes at 37ºC. Human thrombin in 40 mM $CaCl_2$ (Sigma) was then added to the PEGylated Fib to begin Fib crosslinking. The final concentration of TGF was 25 ng/ml; PDGF, 100 ng/ml; Fib, 10 mg/ml; and thrombin, 12.5 U/ml. Monofunctional mPEG-SMB was utilized to test GF affinity to the matrix. TBS was used in lieu of PEG as a control. Gelation occurred at 37ºC over 30 minutes. Gels were incubated in PBS supplemented with 1% antibiotic-antimycotic (Invitrogen) and collected every other day up to 10 days.

Release curves for TGF and PDGF was quantified using purchased TGF ELISA kit (R&D Systems) and PDGF ELISA kit (R&D Systems). TGF bioactivity was analyzed by using the Mv1Lu growth inhibition assay. Gels were formed in trans-wells and exposed to a subconfluent culture of Mv1Lu cells. Proliferation was measured by calcein AM dye (Sigma) uptake and normalized to untreated cells. PDGF migration potential was analyzed using a modified Boyden chamber (Neuro Probe) and CFDA SE cell tracer kit (Invitrogen).

Results / Discussion:

TGF and PDGF release was measured over 10 days from PEGylated fibrin gels. Figure 1 demonstrates that release of TGF from non-PEGylated gels was significantly greater than PEGylated gels from days 2-8 and thus PEGylated fibrin gels can sequester TGF for up to 8 days. Despite observed covalent linkage of TGF to Fib through PEG-$(SMB)_2$, no significant difference was observed in TGF release between mPEG-SMB and PEG-$(SMB)_2$ indicating that the predominant mechanism of TGF conjugation was direct physical affinity with the matrix. Release of TGF is correlated with degradation curves as observed through BCA analysis (results not shown) indicating that TGF release is controlled by matrix degradation. PDGF in all groups was nearly completely released by day 4 demonstrating that PDGF can be entrapped in the gels and released in a short time course.

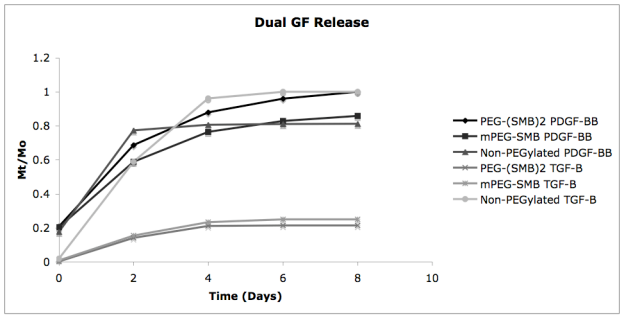

Figure 1: TGF and PDGF release from fibrin gels

TGF bioactivity was analyzed using the Mv1Lu growth inhibition assay. When exposed to TGF, proliferation of Mv1Lu cells is inhibited. Figure 2 shows bioactivity data for only PEG-$(SMB)_2$ gels as representative of all groups. Proliferation of Mv1Lu cells exposed to gels containing GF was significantly inhibited when compared to gels without GF addition. Thus, TGF maintains its bioactivity in both PEGylated and non-PEGylated gels.

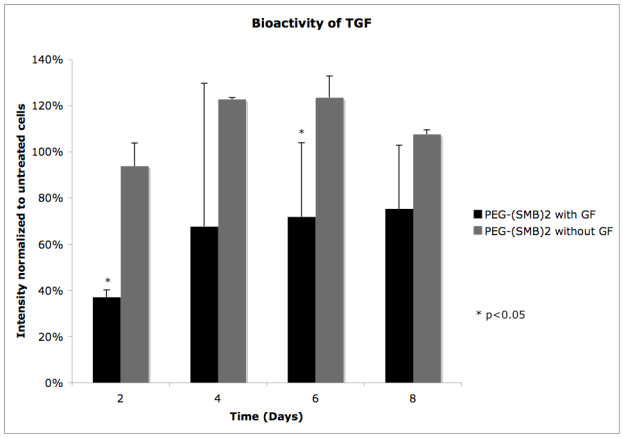

Figure 2: Bioactivity of TGF

Conclusions:

GF can be incorporated into a PEGylated fibrin gel with PEG-$(SMB)_2$ and released at varying rates. PDGF is released within 4 days while TGF can be sequestered within PEGylated fibrin gels for at least 8 days. Bioactivity of TGF is maintained within all fibrin gels, but further research is needed to analyze chemotactic potential of PDGF within PEGylated fibrin gels.

References:

1. Hautmann et al. J Biol Chem 1997;272(16).
2. Hirschi et al. Circ Res 1999; 84(3).