# Sustained Release of Transforming Growth Factor-β1 from PEGylated Fibrin Gels

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**Statement of Purpose:**

Transforming growth factor-beta 1 (TGF-β) has demonstrated ability both *in vitro* and *vivo* to upregulate cell markers of the mesenchyme from bone marrow progenitor cells.[1] The aim of the current study is to obtain sustained release from a fibrin gel by PEGylating fibrinogen with bifunctional succinimidyal α-methylbutanote poly(ethylene glycol) (PEG-$(SMB)_2$) which alters the release kinetics and degradation rate.[2]

**Methods:**

Porcine fibrinogen (80 mg/ml in TBS, pH 7.8, Sigma-Aldrich) was PEGylated with PEG-$(SMB)_2$, 3400 MW (Nektar Therapeutics) in a 1:10 molar ratio. The reaction occurred over 20 minutes at room temperature in TBS, pH 7.6. A 100 ng/ml human TGF-β (R&D Systems) solution in TBS, pH 7.6 was immediately added to the PEGylated fibrinogen at equal volume, and stored at 37 ºC for 1 hour. Human thrombin (25 U/ml in 40 mM $CaCl_2$, Sigma) was then added to the PEGylated fibrinogen and TGF-β solution to begin fibrinogen crosslinking. The final concentration of TGF-β was 25 ng/ml; fibrinogen, 10 mg/ml; and thrombin, 12.5 U/ml. Controls consisted of non-derivatized PEG, 3400 MW (Sigma-Aldrich) with TGF-β, TGF-β without PEG, bovine albumin (Sigma-Aldrich) for TGF-β, and TBS alone.

Gelation occurred at room temperature for 30 minutes. Afterwards, gels were rinsed with DMEM (Gibco) supplemented with 1% penicillin/streptomycin (Invitrogen) to remove any unbound material and replaced with the same media. The media was immediately removed for day zero analysis, and subsequently removed at days 1, 3, 5, 7, and 10 to obtain a release curve.

The amount of TGF-β released was quantified using a purchased TGF-β ELISA kit (R&D Systems)[3]. Briefly, sample solutions were incubated for 3 hours at 37 ºC on well strips precoated with TGF-β receptor type II. Following a wash to remove unbound substances, an antibody for TGF-β conjugated to horseradish peroxidase was added to the wells. After another washing step, a substrate solution initiated color development in proportion to TGF-β concentration. Color formation was stopped with a 2 N sulfuric acid solution and measured with a spectrophotometer set at 450 nm. Samples were compared against known concentrations of TGF-β to quantify the amount of TGF-β released from the gels.

**Results / Discussion:**

Results with a prototype growth factor, stromal cell derived growth factor-1α (SDF-1α), demonstrated the potential for release from PEGylated fibrin gels. SDS-PAGE analysis shown in Figure 1 indicates that SDF-1α covalently binds to PEGylated fibrinogen. Immunoprecipitation techniques using a primary antibody against fibrinogen removed any unreacted or PEGylated SDF-1α. Furthermore, western blotting with an antibody against SDF-1α ensured that only PEGylated fibrinogen with bound SDF-1α was detected. This is indicated in lanes 2-5 of Figure 1.

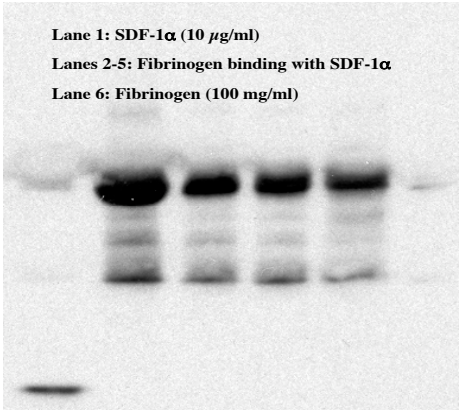


**Figure 1: SDS-PAGE image of SDF-1α binding to PEGylated fibrinogen**

Using an ELISA for SDF-1α, a release curve was obtained throughout the two-week lifetime of the fibrin gel. As shown in Figure 2, maximum release occurred on day 1 followed by a constant rate of release for 10 days. Bioactivity of the released SDF-1α was assessed using a migration assay of murine mononuclear cells (MNC). The chemotactic effect of released SDF-1α was not statistically different to commercially available SDF-1α at the same concentration.

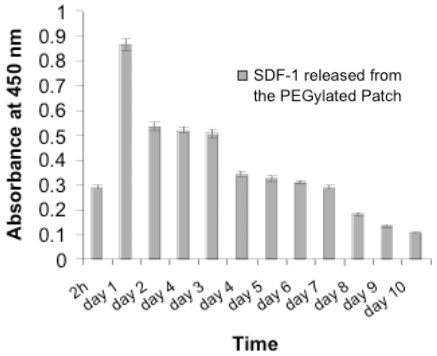


**Figure 2: SDF-1α release from PEGylated gel**

**Conclusions:**

Growth factors can be incorporated into a PEGylated fibrin gel and released at a constant rate when using PEG-$(SMB)_2$ to PEGylate fibrinogen. Furthermore, bioactivity *in vitro* is maintained. Further investigation is required to obtain sustained release of active TGF-β from the PEGylated fibrin gels.

**References:**

1. Hautmann et al. J Biol Chem 1997;272(16).
2. Veronese FM. Biomaterials 2001;22(5).
3. Danielpour D. J Immunol Methods 1993;158(1).