Controlled Release of Bioactive TGF-β1 from Fibrin Gels

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Statement of Purpose: Members of the TGF-β family of growth factors have been identified as key regulators of Mesenchymal Stem Cell (MSC) maturation. In particular, TGF-β1 has been implicated in cartilage and bone development, most likely by inducing the differentiation of MSC into the chondrogenic or osteogenic lineage (1).

In the context of cartilage or bone repair, the use of an appropriate matrix to deliver cells and growth factors as well as to localize and organize the tissue is necessary. Fibrin sealants have been used for cell delivery and shown to be a suitable delivery vehicle for growth factors (2,3). They consist of two human plasma-derived components: (a) a highly concentrated Fibrinogen Complex (FC) composed primarily of fibrinogen and fibronectin along with catalytic amounts of Factor XIII and plasminogen and (b) a high potency thrombin.

The goal of the present study was to assess the ability of different formulations of fibrin gels to deliver added recombinant TGF-β1 and to analyze the biological activity of the released TGF-β1 on Human MSC (HMSC) *in vitro*.

Methods: Eight different formulations of fibrin gels (Tisseel®, Baxter) were prepared using different concentrations of FC and thrombin, from 5-40 mg/ml and 2-250 U/ml, respectively (final concentrations in the gels).

Recombinant (rh) TGF-β1 (15 ng/0.3 ml gel, R&D Systems) was added in the FC component at the time of the gel preparation. All gels were incubated at 37^0C in 5% CO_2 for up to 10 days with standard HMSC growth medium (Cambrex). Culture medium was changed every day and culture medium samples were frozen until tested for the amount of TGF-β1 by ELISA.

In order to test the biological activity of the released TGF-β1, medium supernatant from gels at day 3 was used as culture medium for monolayers of HMSC (Cambrex). Changes in cell morphology up to 7 days were analyzed by light and fluorescence microscopy after staining with calcein dye. Cell proliferation was also analyzed after staining with calcein dye. Statistical analysis was performed using the ANOVA test (with 5% as the level of significance).

Results: ELISA results on the effect of FC concentration on daily TGF-β1 release showed a spike release at day 1 and a significantly higher release with gels containing lower FC concentrations. Also, the cumulative release of TGF-β1 by day 10 was lower than the initial added amount of the growth factor (15 ng) (Fig. 1).

ELISA results on the effect of thrombin concentration also showed a spike release of TGF-β1 at day 1 and a similar release with all thrombin concentrations analyzed except with 250 U/ml showing a significantly higher release of TGF-β1. Similar to the effects of FC, the cumulative release of TGF-β by day 10 was lower than the initial added amount of the growth factor (Fig. 2).

HMSC cultured with medium supernatant from the gels at day 3 (containing released TGF-β1) showed a change in morphology, depicting a more squared-shape than those cultured with medium with no TGF-β1. Cell proliferation was also lower in presence of TGF-β1 released from the gels.

Discussion and Conclusions: This study showed that added rhTGF-β1 in fibrin gels was gradually released from the gels. The release was lower when FC concentrations were high, suggesting an effect of FC concentration on the release kinetic and a binding affinity of TGF-β1 with the FC component of fibrin. This binding affinity was further confirmed by a cumulative release after 10 days that was lower than the initial added amount of rhTGF-β1, for all FC concentrations analyzed. These effects of FC concentration on the release kinetic suggest an intrinsic property of fibrin to reversibly bind TGF-β1. TGF-β1 release from gels with 2, 10 and 50 U/ml of thrombin (with 25 mg/ml of FC) was similar, suggesting that thrombin concentration had a lesser effect. TGF-β1 release was only significantly higher with the highest thrombin concentration (250 U/ml) suggesting an effect of the gel structure, with a more heterogeneous structure and a faster gel degradation with this high thrombin concentration.

The more squared-shape cells after culture in medium containing released TGF-β1 from the gels suggested their differentiation, in parallel to a lower cell proliferation compared to cells cultured in medium that did not contain released TGF-β1. These changes in cell morphology and proliferation demonstrated that the released TGF-β1 from the gels was still biologically active.

Overall, the present study demonstrated that fibrin gels could be considered as a potential carrier system to deliver biologically active TGF-β1 after reversed-binding and in a controlled manner by adjusting the concentrations of FC and thrombin.

References: 1. Centrella M. et al. Endocrine Rev. 1994;15(1):27-39, 2. Cox S. et al. Tissue Eng. 2004;10(5-6):942-954, 3. Wong C. et al. Thromb Haemost. 2003;89(3):573-582.

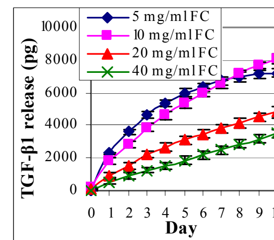

Fig 1: Effects of [FC] on the cumulative release of TGF-β1 from gels ([Th] fixed at 2U/ml)

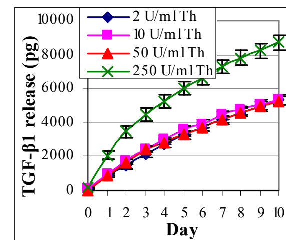

Fig 2: Effects of [Th] on the cumulative release of TGF-β1 from gels ([FC] fixed at 25 mg/ml)