Optimisation of a Fibrin Scaffold for Sustained Release of an Adenoviral Gene Vector

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Introduction: Successful gene therapy depends on efficiency of gene transfer and subsequent level and duration of expression of the therapeutic gene [1]. However, the major limitation of adenoviral vector-based gene delivery is the transient nature of transgene expression [2]. It is our hypothesis that a biodegradable fibrin scaffold will prevent run off of vector solution and provide an efficient system of prolonged gene expression. The specific aims of our investigations are:

- To characterise the release rate of an adenoviral vector from fibrin in order to optimize the fibrinogen / thrombin concentration.
- To assess binding of the virus to fibrin, in order to determine the mechanism of diffusion of the viral vector.
- To determine whether an adenoviral-binding site can be identified on the fibrinogen molecule.

Methods: Fibrin scaffolds of varying concentrations were fabricated containing $1X10^8$ adenoviral particles encoding β-Galactosidase (βGal), and covered in PBS solution. At fixed time points, the elution fluid was collected and replaced. Collected elution fluid was used to transfect human fibroblasts (HFFF), and cells were subsequently stained for expression of βGal, with XGal stain. The area fraction of stained cells was quantified for each group to give an indication of the amount of viral particles present in elution fluid, which had diffused from fibrin. In order to assess binding of adenovirus to fibrinogen, an ELISA plate was coated with 6µg of fibrinogen and blocked with 1% fish gelatin. Serial dilutions of adenoviral particles were added to the immobilised fibrinogen, and bound adenoviral particles were detected with rabbit anti human adenovirus type 5 (AbCam). Inhibition of binding to immobilised fibrinogen was tested by pre-incubating adenovirus with increasing concentrations of soluble fibrinogen. In order to determine a possible binding site, thrombin was added to immobilised fibrinogen in order to expose binding sites on the activated fibrinogen and to create a pseudo fibrin gel in the ELISA plate.

Results and Discussion: Each fibrin formulation showed a distinct elution profile (Figure 1). A concentration of 60mg/ml fibrinogen and 4IU thrombin showed sustained release at 192 hours. Positive binding between adenovirus and fibrinogen was seen (Figure 2). Inhibition of binding was observed when adenovirus was incubated with increasing fibrinogen molecules. Enhanced binding to the fibrinogen / thrombin system was observed, reaching saturation in advance of the fibrinogen coated assay (Figure 2).

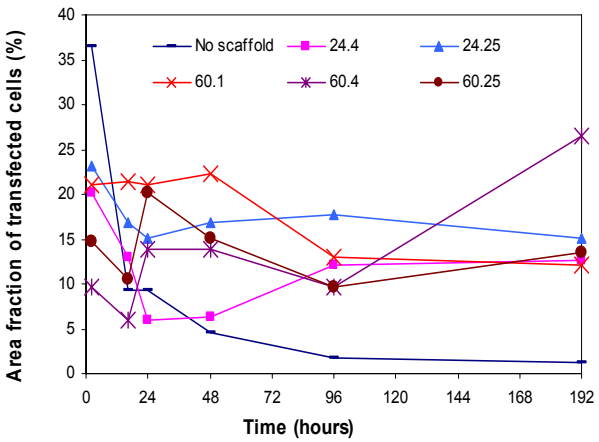

Figure 1: Graph showing the rate of release of adenoviral particles released from fibrin.

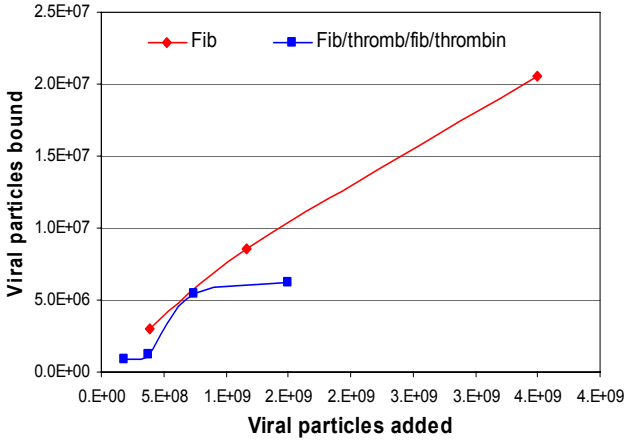

Figure 2: Graph showing positive binding between adenovirus and fibrinogen alone (red), and fibrinogen / thrombin layer (blue)

Conclusions: Fibrin concentration is a determinant in the rate of release of adenovirus. 60mg/ml fibrinogen and 4IU thrombin shows sustained release up to one week. There was binding detected between adenovirus and fibrinogen and the binding site may be exposed upon addition of thrombin.

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References:

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