Dilution of Fibrin Glue Affects Release of Adeno-Associated Virus: 
Implications in Gene Delivery for Cartilage Tissue Engineering

INTRODUCTION:
Limited repair potential of the articular cartilage contributes to the 
development of debilitating osteoarthritis and is a great clinical 
challenge. Although clinical strategies for management of cartilage 
pathology are numerous, results have been equivocal and no single 
approach has been proven to be superior [1]. Therefore, the utilization 
of “gene-loaded” biodegradable scaffolds that release nucleic acids 
encoding for chondrogenic growth factors or inhibitors of cartilage 
catabolites is of interest for cartilage tissue engineering. The long 
term release of bioactive factors can better promote local cell 
proliferation and differentiation via sustained delivery to the cells, 
overcoming the quick dilutions of these factors and the need for multiple interventions. 
Viral vectors that have been used in preclinical setting and in human 
clinical trials include adeno-associated virus (AAV) [2, 3].

Fibrin glue (FG) is a biomaterial widely used in clinical practice and 
tissue engineering applications. It has been shown that dilute FG hydrogels produce a more open fibrin network compared to undiluted FGs [4]. FG can also act as an efficient scaffold for nucleic acid vector delivery [5]. We investigated the effect of different FG dilutions on the delivery of AAV-GFP in vitro and in vivo. The aim of this study is to test the hypothesis that diluted FGs will result in more release of viral particles, which could be advantageous for effective therapy using AAV.

METHODS:
Commercially available Tisscel® human fibrin sealant (Baxter) was used to make the FG hydrogels. Four different fibrinogen concentrations (25%, 50%, 75%, and 100%) were prepared by dilution with PBS. FG hydrogels were formed according to the fibrinogen concentrations. To evaluate clotting time, change in turbidity of the FG mixture was measured by a UV-Vis spectrophotometer (550-nm), immediately upon thrombin activation [6]. Scanning Electron Microscopy (SEM) was performed to determine fibrin fiber thickness and pore size (Metamorph 7.6.2). Degradation of the FG hydrogels in PBS was measured every other day as wet weight fraction \([W_t - W_0/W_0]\).

For the in vitro transduction assay, 6.25 x 10^10 vg/mL of AAV-GFP was added to the fibrinogen-thrombin solution and immediately molded in uncoated 24-well plate (WP) inserts (8-μm pores). Each day, fresh HEK-293 cells were seeded and incubated for 24 hours with the inserts. Transduction efficiency, as the percentage of GFP-positive cells, was quantified using flow cytometry.

Pilot in vivo study of AAV release and transduction into the joint tissue was performed by creating an osteochondral defect on the femoral cartilage of Nude rats. 6.25 x 10^11 vg/mL AAV-Luciferase (AAV-Luc) was injected into the knee where AAV-Luc-loaded 50% FG was implanted (Fig. 3).

RESULTS SECTION:
Characterization of FG hydrogels showed that the clotting time of the 100% FG was significantly longer than those of the diluted (25%, 50%, and 75%) FGs (Fig. 1a). The fibrin fiber thickness of the scaffolds, assessed by SEM, increased with increasing FG concentrations (Fig. 1b). The increases in fiber thickness were significant for each levels of the FG concentration. The pore size of the 100% FG was significantly smaller in diameter compared to those of the diluted FGs (Fig. 1c). Taken together, these data indicate that 100% FG formed a denser network of fibrin scaffold, which explains the longer clotting time. In contrast, diluted FGs had a more open fibrin network, which are reflected in their shorter clotting times.

Analysis of FG in vitro degradation in PBS showed a biphasic pattern of degradation (Fig. 2a). The first spike in degradation occurred at day 2 for low (25%) and high (100%) FGs, followed by a second spike at day 8. For 75% and 100% (high) FGs, the two spikes in degradation occurred at days 6 and 11. In addition, low FGs displayed a significantly higher rate of degradation than high FGs. This biphasic FG degradation coincide with the two spikes in the percentage of GFP-positive cells (Fig. 2b), suggesting that AAV-GFP was released in a biphasic burst fashion. Similarly, FGs of lower concentrations had significantly higher transduction efficiency than FGs of higher concentrations.

Using the information obtained from in vitro characterizations, 50% 
and 100% FGs were used for a pilot in vivo study. AAV-transgene expression was detectable starting at day 9-post surgery, only on the left knee where AAV-Luc-loaded 50% FG was implanted (Fig. 3).

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