Aptamers Assisted Controlled Growth Factor Delivery Enables Self-Organizing Microvasculature within 3D Microenvironment

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Statement of Purpose

Self-organizing vasculature is essential for the success of engineered tissues. Modulating spatiotemporally controlled growth factor's availability within engineered tissues could help in guiding the developing vasculature. However, conventional approaches for growth factor delivery often relies on their immobilization or coupling within the polymer matrices (via linker proteins or peptides). Even though stable release rates have been achieved, the approach imparts limitations while upscaling with high specificity for multiple growth factors delivery. To this end, the present study employed DNA based aptamers, that are affinity ligands designed to recognize proteins with high affinity and specificity,[1] for harnessing growth factor availability within polymer matrix to guide developing microvasculature.

Aim

The aim of the study is to develop vascular endothelial growth factor ($VEGF_{165}$) specific aptamer-functionalized hydrogel for achieving spatiotemporally controlled VEGF delivery and harness its availability for guiding self-organizing microvascular networks within 3D microenvironment.

Materials & Methods

The aptamer-functionalized hydrogel was prepared via photo-polymerization of gelatin methacryloyl (GelMA) and acrydite functionalized aptamers having DNA sequence specific for binding to VEGF. Visible light photoinitiator, tris-dichloro-ruthenium (II) hexahydrate with sodium persulfate were used. For patterning, 3D bioprinting was employed where two bio-inks were prepared (i) with aptamer-functionalized hydrogel & (ii) GelMA + fluorescent blue microbeads (control). For bio-inks, the pre-polymer solutions were mixed with human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs). The construct were 3D bioprinted as lines of aptamer-functionalized bio-ink next to control GelMA lines, making an interface. Followed by photo-crosslinking and VEGF loading for 1 hr. It was expected that the 3D bioprinted aptamer lines would be able to sequester VEGF from the culture medium, compared to GelMA lines. Furthermore, triggered VEGF release efficiency was studied by adding the complementary sequences (CS) at specific time-points (using ELISA assay) and its effect on microvascular network formation in 3D (cell viability & immunostainings).

Results

The results obtained from VEGF ELISA experiments confirmed triggered release of VEGF from the aptamer-functionalized hydrogels in response to CS addition. Without CS addition, these hydrogels could sustain a controlled release until 10 days. Furthermore, the bioprinted aptamer-functionalized hydrogels showed high cellular viability and ability to guide microvascular network formation (by HUVECs and MSCs) only within the aptamer-functionalized regions of the pattern, and not in GelMA regions, after 10 days of culture (see Figure 1). However, differences in the microvascular organization was observed in the samples with triggered VEGF release on day 5, compared to the samples without the VEGF release. These observations altogether confirmed the ability of patterned aptamer functionalized hydrogels in controlling self-organizing microvascular networks.

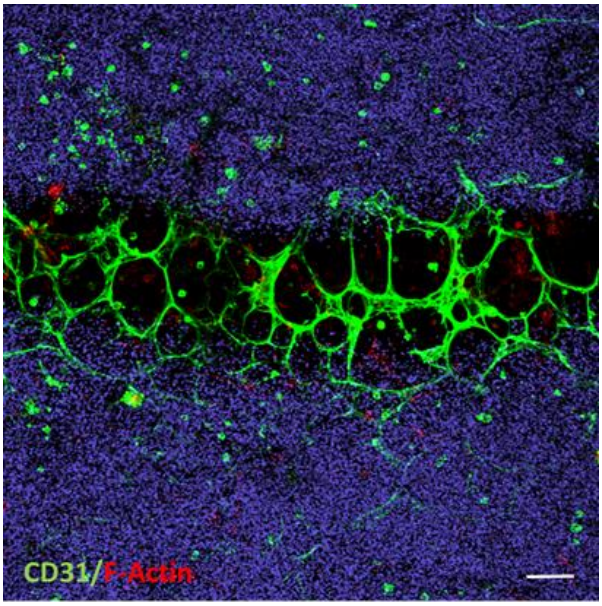

Figure 1: The guided self-organizing microvascular network formation confined within the VEGF–loaded 3D bioprinted aptamer regions. Blue fluorescent beads indicates GelMA region. The image is a maximum projection of confocal z-stack. Scale bar is 100 microns.

Conclusions

The present study confirms the potential of 3D bioprinted aptamer-functionalized hydrogels in guiding self-organizing microvascular networks within 3D microenvironment and their ability to spatiotemporally controlled VEGF bioavailability.

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