# Hyaluronic Acid Binding Peptide Mediated Directed ECM Assembly to Prevent Fibrotic Capsule Formation

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## Statement of Purpose

Implants have revolutionized the medical field. They play pivotal roles in the body depending on their function. However, several case reports indicate implant failures due to various causes including fibrotic capsule formation (FCF). Fibroblasts are thought to be the regulators of deposition of excess of the ECM (in particular dense collagen fibrils) during FCF that form around foreign bodies (Noskovicova et al., 2021).

Hyaluronic acid (HA) is a glycosaminoglycan in the ECM that is known to regulate collagen organization. This is a function of HA that could be utilized to address fibrotic capsule formation. HA binding peptides are a class of bioactive sequences that are able to form strong bonds with HA thus retain HA in the sites they are present (Singh et al., 2014). In this study, a new mussel-inspired immobilization strategy based on covalent catechol-mediated adhesion was developed, in combination with a layer made up of laccase from *trametes versicolor* enzyme in order to conjugate HABP on surface. Using this strategy, the synthetic HABP was anchored over a DOPA (3,4-Dihydroxy-L-phenylalanine) on titan and gold surfaces, locally binding and concentration the lubricant. We propose that this will decrease implant failure rates related to fibrotic capsules and improve implant lifespan within the body.

## Methods

**Peptide synthesis and Material Surface treatment:** HABP and control peptides containing DOPA groups were synthesized using our earlier protocols (Corrales-Ureña et al., 2020). **Primary Cell Culture and Cell derived ECM deposition:** Human primary dermal fibroblasts (HDFas) were cultured in DMEM (Dulbecco's Modified Eagle's Media) media with fetal bovine serum (FBS), and penicillin-streptomycin. Fibroblast derived matrices were generated by incubating cells growing on Ti surfaces for 9 days and further decellularizing with extraction buffer. **Cell Viability and Cytotoxity of synthesized peptide molecules** PrestoBlue and Live/Dead cell viability assays were performed to measure the cytotoxicity of the HABP solution at varying concentrations. 10% PrestoBlue solution in media was added to the cell culture wells and fluorescence intensity change was detected after 2 hours analyzed with a plate reader (SpectraMax i3). Calcein-AM (1:1000 dilution) and Propidium Iodide (1:5000) was added onto cells at 37 ˚C and incubated for 15 minutes followed by immediately imaging with a fluorescence microscope (Nikon Ti-S). **Immunostaining and SEM Imaging** Presence of collagen depositing myofibroblast formation was performed using immunofluorescence labeling against α-smooth muscle actin (α-SMA). Fluorescence labeled α-SMA in cells were imaged using fluorescence microscope (Nikon Ti-S). FDMs were fixed using Karnovsky Fixative and samples were dehydrated using an ethanol gradient and hexamethyldisalizine (HMDS) treatment and imaged using Scanning electron microscopy (SEM) (Axia ChemiSEM). Physical properties of collagen fibrils (alignment, density) analyzed using NIH ImageJ.

## Summary of Results

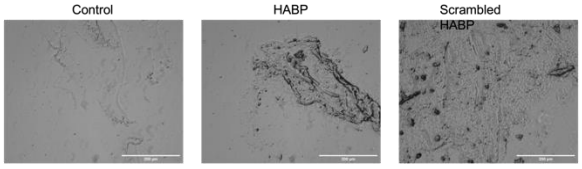


*Figure 1 Binding of HA on HABP treated surfaces using bright field microscopy. Scale bar represents 200 μm.*

Staining with methylene blue found that HA was able to bind to HABP better than Scrambled HABP and the amine coatings. PrestoBlue results also indicate that fibroblasts do not have cytotoxic responses towards soluble peptides.

## Conclusions Reached

Coating of TiO2 surfaces with HABP could potentially initiate HA deposition and further diminished collagen accumulation.

## References

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