Transcytotic Intercellular Gelation Enables Cell Spheroids

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Abstract

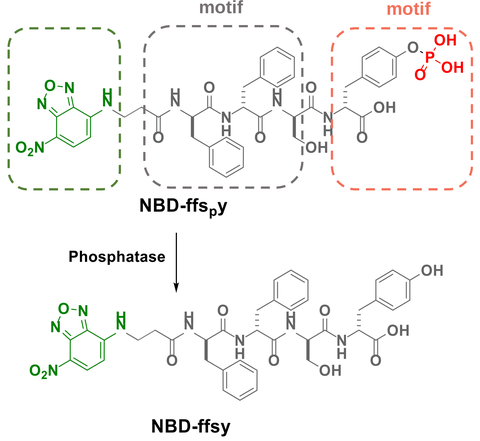
Here we report enzymatic noncovalent synthesis of self-limiting peptide nanofibers in the intercellular space for generating cell spheroids. We used cryo-EM to determine the atomic structure of helical nanofibers self-assembled from D-peptides and used fluorescent imaging to show that endo/exocytosis of enzyme-responsive D-peptide assemblies results in intercellular gels that enable cell spheroids. Specifically, D-phosphopeptides self-assemble to form nanoparticles to undergo endocytosis. The nanoparticles, being resistant to proteolysis and partially dephosphorylated, go through exocytosis to the cell surface and turn into helical nanofibers, which act as the artificial matrices of intercellular gels to induce cell spheroids.

Background

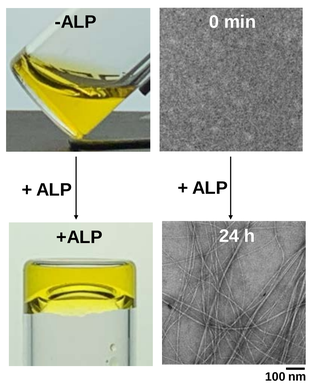
- Current methods to generate cell spheroids were tedious and time-consuming.
- Lack of materials that mimic the highly dynamic and complex extracellular matrices (ECM)
- Supramolecular peptide assemblies depend on noncovalent interactions that are inherently dynamic and context-dependent.

Results

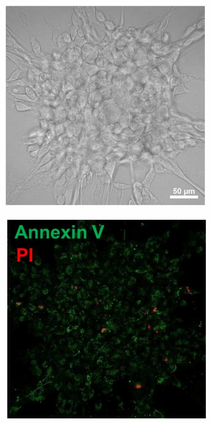
Molecular design



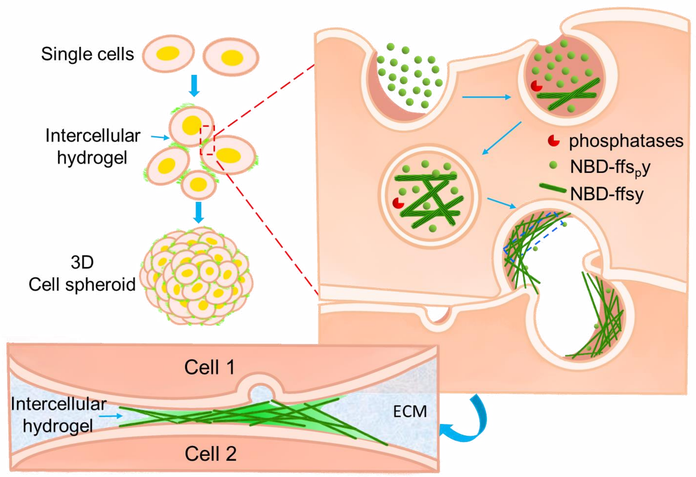
Hydrogelation and morphological change



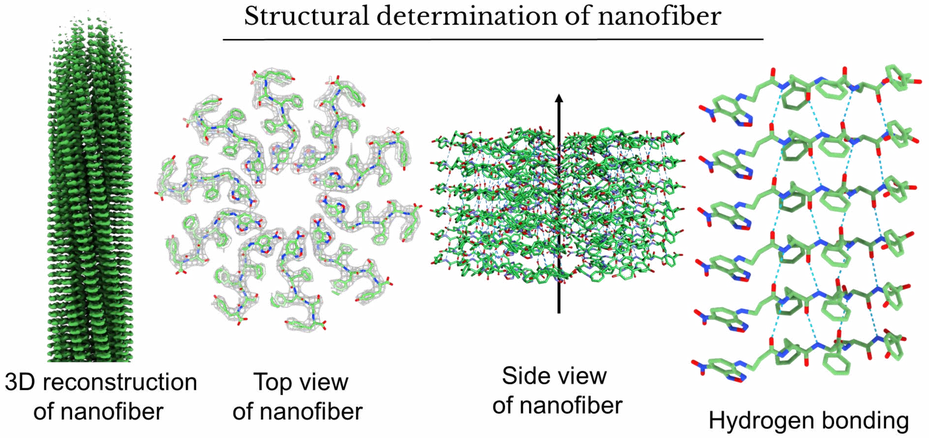
Generation of viable spheroids



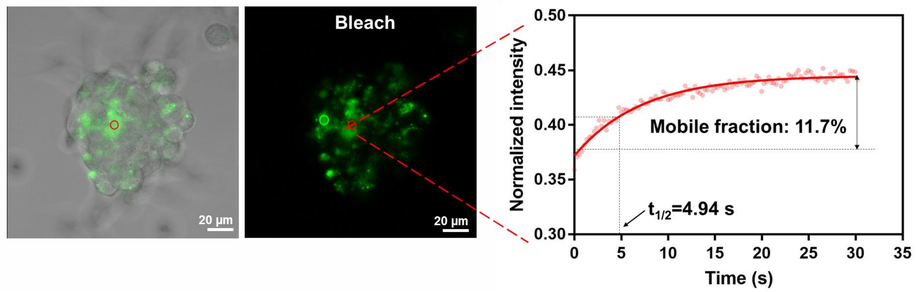
Intercellular hydrogelation made of self-limiting nanofibers to induce spheroids



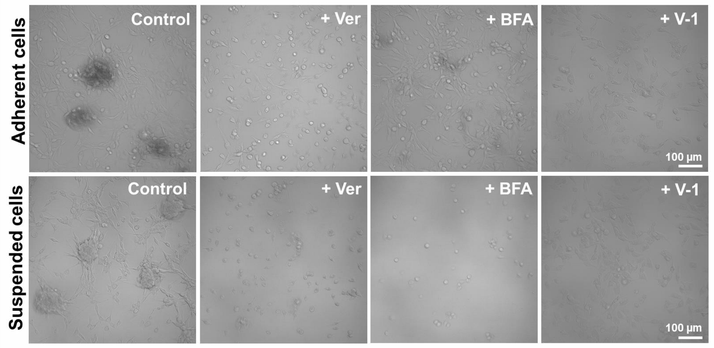
Structural determination of nanofiber

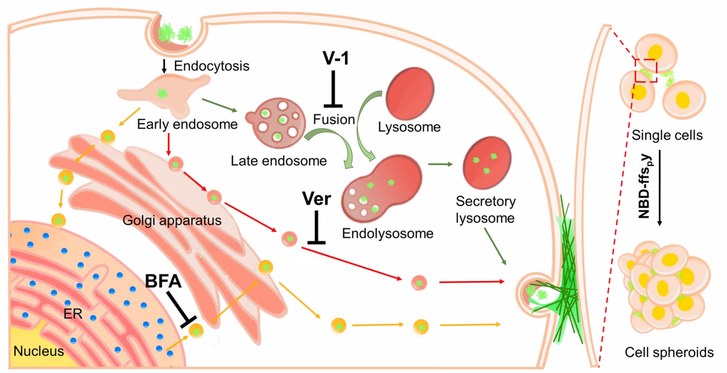


In situ hydrogelation confirmed by FRAP



Transcytosis of the nanofibers to the cell surface





Conclusions

- Self-limiting D-peptide nanofibers formed during endosomal trafficking lead to intercellular hydrogels for fast spheroid formation.
- The structures of the nanofibers in complex extracellular matrix were determined.
- Inhibiting exocytosis abrogate spheroid formation

Acknowledgement

This work was partially supported by NIH Grants CA142746, GM122510 (E.H.E.) K99GM138756 (F.W.), and NSF Grant DMR-2011846.