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Nanobead Characterization of Self-Assembling Peptides

RADi6 and RIDI2
Abstract
I would also like to thank Jessica Dai (graduate student) for helping me in the laboratory.
with the opportunity to work on this project and for his guidance throughout my research.
I would like to thank my thesis supervisor, Dr. Shuang Zhang, for providing me

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Frequently residual growth factors and undetected contaminants of non-guaninized
derived from animals, while the length scale is that of the relevant cells, there are
allowing important biomolecules to quickly diffuse away. Regarding biomaterials
approximately 1,000-10,000 times larger than the biomolecules of interest, thereby
of disadvantages, the formation of pores between the fibers (~10-200 μm) are
little gained advantage over the conventional two-dimensional surface. Adding to the list
therefore, the "matrix" is still a two-dimensional surface and thus the cells attached have
consisting of microfibers ~10-50 μm are similar in size to that of the cells of interest.
Poly(sodiumphosphate and Integra®). However, the synthetic processed polymers
addition to biomaterials derived from animals (e.g., collagen fibrils, polymers
attempts have been made to fabricate these scaffolds using polymers/copolymers in
cultures has resulted in the ability to create a synthetic extracellular matrix (ECM).

The desire for scientists to go beyond the two-dimensional environment of cell

can improved scaffolds be made by using "gel" peptides仅有"?
are already known to individually self-assemble into nanothreads and form scaffolds. But
formation and scaffold organization. The two peptides used, RADII-1 and RADIA16-1.
peptides will provide the next step in understanding multiple cell type neighbors.
work has only introduced one peptide (i.e., RADIA16-1) into the scaffold. The use of two
formation using a mixture of two different self-assembling peptide solutions. Previous.

The length of this investigation analyses the ability to control the length of their

1. Introduction
The nanometer-scale scaffolds act as a template for materialization. The template is then fabricated to bind with metal nanoparticles via conventional peptide chemistry, resulting in fabrication of conducting protein nanowires. The functionality of the peptides can be assessed by providing greater accuracy of in vivo behavior assays and advances in research is needed in order to achieve higher order architecture with multiple cell types.

Furthermore, all these studies involve single cell type scaffolds. Further assembly of a‑sheet peptide and the controls of the self-assembly zones. Further work has produced insightful into the self-assembly patterns. A local gradient while allowing the slow diffusion of biomolecules through the membrane into the ECM in that it surrounds the cells and physiological pH or NaCl concentration. The three-dimensional structure that can be printed or homogenized and mass produced; and they can be controlled at the nanoscale level; the three-dimensional scaffolds with defined compositions, these nanotubes build a line between a new material that can be also be chemically synthesized to mimic the ECM and incorporate ligands for specific cell receptors; they can be designed self-assembling peptide scaffolds. Zhao and coworkers have demonstrated synthetic biological materials with defined compositions at the nanoscale, e.g., molecular.

In response to these problems, Zhao and coworkers have proposed using polymers or animal-derived materials scaffolds for a well-controlled study. One can see that these obstacles present a difficulty in using synthetic
Formation and scaffold organization.

This technology is to gain further insight into the properties and control of another nanowires, such as biosensors, but the first step towards an effective implementation of removed to form a conducting nanowire. One can envision many applications of
peptides, self-assemble as a result of the "hydrophobic effect" and perhaps intermolecular

attractive diameter of 30-50 nm with a helical twist. These type-I amphiphilic
charged head and hydrophilic tail are able to form nanosomes and nanovesicles having an
analogous to surfactants and biological lipoicids, short peptides consisting of a
particular interest.

However, for this investigation type-I and type-IV peptides of
molecular cavity). However, for this investigation type-I and type-IV (molecular capsule), and type-A
(molecular switch), type-III (molecular electro) type-IV (molecular legs), type-II

synergistically studied by Shinaune et al. type-I (molecular legs), type-II

developed, few have been thoroughly studied. He has been
replacement of cartilage in joints. Of the many self-assembling peptide systems
mimic the body's extracellular matrix (ECM) and to signal chondrocyte growth for the
developed in the laboratory. In one case, synthetic self-assembling peptides are used in

literature provides numerous examples of synthetic self-assembling molecules (SAMS)
through a number of non-covalent interactions. While intriguing in nature, recent

and thus shift equilibrium conditions into structurally well-defined and reactive assemblies
assembly describes the spontaneous organization of molecules under thermodynamic

self, the answer, self-assembly—building from the bottom-up, self-
facilitate materials with phenomenal properties, i.e., sea shell biomaterialization, spider

Several attempts have been made to understand and mimic nature's ability to

2.1 Self-Assembling Peptides

2. Background
calcify efforts in tissue-engineering research. Provide an alternative to the two-dimensional in vitro environment and will continue to

neutrophilic growth and synapse formation, in addition to high-potential Sephades has been proven to attach to mammalian cells and in another case provided a scaffold for cell adhesion motif RAD that is found in many ECM proteins. The RAD16 scaffold cell structure. Relevat to this thesis, the peptide sequence RAD16-I, is of the type-I

hinges allow for the "sticking of Lego blocks" (mamillary formation), resulting in beta-holes similar to Lego blocks. These adhesion + charged regions (pegs and pegs and Ty peptide and have all the active phosphor and hydrophobic regions. Accine as pegs and pegs another self-assembling peptide able to form nanoholes is the type-I peptide.

Another self-assembling peptide able to form nanoholes is the type-I peptide.

To continue this brief insight into the topic:

The applications for self-assembling peptides will quickly expand as ongoing research in organic material, therapy leaving behind a hollow metallic wire at the sub-micron level, nanohole formation is followed by metallization and then dissolving or dissolving of the issue-confinement, i.e., biomimeticization, and as template for nanowires. In one case, hydrogen-bonding. Specifically family peptides are intensely being implemented in
Hydrophilic cavities in which clusters of water can reside. The allowance for aqueous
membranes with a high aspect ratio and surface area. These nanotube networks have
amphiphilic molecules' ability to trap water stems from its ability to self-assemble into
and R1D12 form a hydrogel matrix when water content greater than 99% (v/vol). The
All low milli-molar concentrations in salt-free conditions, the separated R1D16

2.2 Hydrogels and Scaffolds

The investigation of the R1D12 properties, peptide sequence has not been extensively investigated, but this thesis will continue to
due to the decrease in flexibility needed to sustain the beta-sheet structure. The R1D12
length of the sequence effects entropy; the longer the sequence the greater loss in entropy
increased because the enthalpy penalty for bringing unstructured is also increased. The
amino acid in a more hydrophilic solubilization amino acid the driving force for assembly is
from hydrogels, but the hydrophilic amino acid is now insolubilized. By changing the amine
solubilization-aspartate, R1D12-1. The sequence is very similar to R1D16 in that they both
Another important type-1 peptide sequence is the repeated sequence, Arginine-

Figure 1. Molecular model of R1D16-1 peptide: The hydrophilic side chains of
arginine and aspartic acid are on the upper side and the hydrophobic alanine side
chairs are directed toward the bottom side of this model.
response. Many animal-derived biomaterials carry pathogens, e.g., transmissible
with the host, thereby allowing cell growth and adhesion without an immunological
These scaffolds are designed to operate in vivo and will need to be biocompatible

nanometer-sized, allowing for behavior similar to the natural ECM.

However, the self-assembling peptides have diameters and pores on the order of
ECM (in addition to having large pores that may let biomolecules escape at will.

Therefore, they do not possess a three-dimensional environment (dissimilar to the
polymers have diameters similar in size to most cells ~10-50 μm) and pores ~10-200
thin may be too great, resulting in a less effective scaffold. Many processes synthetic

If the pores are too big then important biomolecules may be released and exchange of
material and biomolecules through the network in addition to the erosion of the scaffold
be exchanged through a porous network. Pore size is integral in the migration of many
atoms to form a scaffold with local microenvironments where nutrients and waste can
be addressed when aiming for the use of synthetic materials for in vivo use. Nanofibers self-

use as issues-confining scaffolds. However, there are some issues that need to be
achieved that are analogous to an extracellular matrix and therefore, very desirable for

The nanofiber formation of the hydrogels provides a three-dimensional
affords hydrogel and scaffold properties.

Research needs to be completed in order to fully understand how the choice of peptides
formation and scaffold organization of peptide hydrogels. However, further
sequence length, and the ionic environment all play an important role in the nanofiber
into the human body. Previous research has shown that the amino acid sequence,
materials to reside within the matrix is key to an effective implementation of scaffolds
Ideal scaffolds have shown biocompatibility of the scaffold in an animal system. One scaffold is made up of amino acids, which are natural to the human body. One problem is also inflammation due to degradation of scaffolds is avoided because the scaffold is made of self-complementary oligopeptides with an RGD motif found in many ECM proteins. To get around this obstacle, the nanoparticles are...
Ratios being R1D2 (%) to R1D6 (%), respectively.

Mixtures were made of 20/80, 40/60, 60/40, and 80/20 with the
(see separate sections). Mixtures were made of further characterization experiments

together and then dilution with Milli-Q water for further characterization experiments

R1D6 and R1D2 peptides were made by mixing aliquots of both stock solutions

further characterization experiments (see separate sections). Solutions containing both

stock solutions were diluted with Milli-Q water to different concentrations for
correspond to concentrations of 6.4 mM and 6 mM for R1D2 and R1D6, respectively.

Peptide stock solutions were made at 1% with Milli-Q water. Solutions of 1.0% with

\[ \text{R1D2:} \text{AcN-RIDIRIDRIRIDR-CONH}_2 \]
\[ \text{R1D6:} \text{AcN-RADPARADPARADPARADPARAD-CONH}_2 \]

and included:

commercially synthesized and purified (SYGEP Corp, Dublin, CA, www.sygep.com)
determine the properties of the peptides in solution. The peptides used were

The experimental procedures were all performed on dissolved peptide powder in

3.1 Peptide Solutions

3. Methods
Krom-wipes and any remaining liquid on the surface was allowed to air dry.

Brushing the surface 200 µl of Mili-Q water. The edges of the sample were dried with
prepared by placing a 1 µl aliquot on a freshly cleaved mica surface, followed by entirely
helpful. Solutions were diluted to 0.6 M or 0.1 M for AFM imaging. Samples were
resolution of 51.2 x 51.2, with increasing brightness corresponding to an increase in
proportional gain approximately twice the integral gain. The images produced have a
of 1.5 Hz, resonant between 0.3-0.5 V, integral gain between 0.3-0.5 V, and a
Instrument (operating in tapping mode at a tapping frequency of ~60-75 KHz) scan rate
Topographic images were taken with an AFM (Nanoscope IIIa, Digital

3.2 Atomic Force Microscopy (AFM)
4.2 Discussion

R&D2 solution the max length seen was ~700nm. Some occasional micron or greater length fibers present, whereas, in the 100% RAD16 solution mixes with R&D2 solution, nother.

4.1 Results

4 Characterization of RAD16 / R&D2 Nanohubes
This would still need to be observable at the nanoscale to be effective. Before or after fiber formation, the peptide has been mentioned by some, but affecting the formation of the short RAD16 fibers in addition to whether hinderance occurs to prevent fiber formation. There are also questions of whether RAD16 forms in relation to determine which peptides are forming fibers and how they are interacting with each other, formation. There needs to be an advanced visualization technique (nanoscale) able to form a microfiber at the moment now the addition of RIDIZ is hindrance another.

4.3 Future Considerations

Needed to make a conclusion, type of peptide before interaction with its own kind. However, further experiments are was more dissimilar, then RAD16 probably will not interact as readily with a different percentage of RIDIZ suggested that if it does not completely effect RAD16 elongation, percentage mixtures, the RIDIZ solution is interacting with RAD16 and inhibiting fibers then there would have been more fibers on the microscale in the higher RIDIZ affected by RIDIZ. Presumably, if RIDIZ did not play a role in the formation of RAD16 the network of shorter fibers. These long fibers RAD16 fibers that have not been formation of RAD16; however, these are still accidental fibers over a microscale long within...
with other self-assembling peptides.

AFM of other mixture concentrations of RDI2:RADI2; mixtures of RADI2 and RADI2 arrangements of nanoobjects; cell attachment to the scaffold followed by cell proliferation; changes in the aqueous environment (cells, exogenous materials, etc.); controlling the size. Additional experiments could also include and are not limited to the following:

This would be helpful in characterizing the scaffold and nanoobject structure, i.e., pore experiments such as transmission electron microscopy or scanning electron microscopy.

Other properties of the hydrogel may be understood through additional
them as an effective higher order architectural biomimetic.

into these self-assembly phenomena, a greater understanding is still needed in providing
nanotherapeutic properties and organization. While this thesis provides more insight
of nanotherapiers. The next step is to further explore methods of controlling
shown that an assembly of a multiple cell-lype material resulted in a heterogeneous mixture
specifically desired macroscopic, nanoscopic, kinetic, and thermodynamic properties. It is
sages shed light on controlling peptide nanotherpa formation in the hope of developing
leaves among nucleuses. For these and their ability to form nanotherpa, preserving the possibility of a template for nanowires. These nanocell
issue engineered. Self-assembly phenomena are also being investigated for their ability
networked structures for use as a biomaterial, specifically three-dimensional scaffolds for
peptides show a promising future as a new method for forming

5. Conclusions
References
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<tr>
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<td>1564 ± 402</td>
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</tr>
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Table 1: Number Length of RID12 and RID12 Peptide Solutions

Appendix
Figure 2: AFM images of RID12/RAD16 mixture at various compositions.
Figure 3: AFM images of RID12/RAD16 mixture at various compositions.
1 μm, 617±338 nm (2) RID12/RAD16 (80/20%), 6 μm, 463±304 nm

To right: Average fiber length and standard deviation of (1) RID12/RAD16 (60/40%).

Figure 4: AFM images of RID12/RAD16 mixture at various compositions. From left...