UNIVERSITY OF WATERLOO
FACULTY OF ENGINEERING

Notice of PhD Oral Defence

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DEPARTMENT: Chemical Engineering

THESIS TITLE:

Self-Assembling Peptide-Carbon Nanotube Dispersions and Hydrogels for Tissue Engineering and Biosensor Applications

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The thesis is available for overnight perusal and may be signed out until the defence date.

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THESIS ABSTRACT ATTACHED

Abstract

Carbon nanotubes (CNTs) are attractive functional materials for use in a broad range of fields due to their unique mechanical and electrical properties. However, their hydrophobic nature is a major problem for some of these applications. Several approaches such as dispersing them in organic solutions and covalently or non-covalently modifying them have been developed to make CNTs usable for desired applications. Since organic solutions can be problematic for bio-applications and covalent modification can introduce defects into the CNT structure (responsible for its unique properties), the approach of making non-covalent modification is more promising. Different types of polymers and surfactants have been used so far in this way. In the past two decades, self-assembling peptides have emerged as promising functional nanomaterials capable of use for different bio-applications. Employing biocompatible self-assembling peptides for CNT dispersion not only removes the first obstacle to use CNTs in solution, but also can result in a new class of hybrid nanomaterials benefitting from the synergistic effects of peptides and CNTs. To the best of our knowledge, this is the first work reporting the dispersion of CNTs using β-sheet-forming self-assembling ionic-complementary peptides. Also this is the first study on the application of peptide-CNT hybrid dispersions and hydrogels for biosensor and tissue engineering applications.

This thesis focuses on the modification of CNTs with self-assembling peptides, characterization of the resulting hybrid dispersions and their application for biosensor development and scaffolding for tissue engineering and cancer spheroid studies. In particular, the study includes the following topics: (i) characterization of the dispersions of multi-walled carbon nanotubes (MWNTs) modified with EFK16-II peptide, (ii) AFM characterization of dispersions of single-walled carbon nanotubes (SWNTs) modified with EFK8 peptide, (iii) formation of hybrid EFK8-SWNT hydrogels, (iv) application of the hybrid EFK8-SWNT dispersion in electrode modification and design of a hemoglobin biosensor, and (v) application of the EFK8 and EFK8-SWNT hydrogels as scaffolds for tissue engineering and 3D cancer cell spheroid formation.

First, we have shown that by non-covalently modifying MWNTs with the ionic-complementary peptide EFK16-II, very stable dispersions of MWNTs can be formed due to the electrostatic repulsion between self-assembled peptides on the MWNTs. Zeta potential and DLS measurements indicated that as the pH diverges from the isoelectric point of ~ 6.7 for EFK16-II, the repulsion between the particles increases and their resulting sizes decrease. AFM, SEM and TEM studies revealed a uniform

distribution of individual modified MWNTs. Finally, tissue culture plates treated with these hybrid dispersions were found to have enough biocompatibility for cell attachment and growth.

In the next step, EFK8 peptide, a shorter version of EFK16-II, was used to disperse SWNTs in water. Scanning probe microscopy (SPM) techniques based on nano-mechanical measurements and electric force microscopy (EFM) were used to gain a deeper understanding of the interaction between nanotubes and peptides. SPM images revealed that EFK8 fibers wrap around SWNTs and render their outer surfaces hydrophilic which enable their dispersion in water. Also it was shown that the hybrid dispersions can form uniform composite EFK8-SWNT hydrogels upon adding solutions containing a small amount of salt.

In the third part of the study, the application of the hybrid EFK8-SWNT dispersion was investigated for immobilization and direct electrochemistry of hemoglobin (Hb) on a glassy carbon electrode (GCE) as well as the efficacy of this platform for making a hydrogen peroxide (H_2O_2) biosensor. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) experiments showed that the presence of SWNTs in the modifying peptide layer on GCE significantly enhances the electrochemical response of the electrode. Furthermore, this response was further increased as more layers of the EFK8-SWNT dispersion were applied. The next step was to immobilize hemoglobin on the electrode by casting. The effectiveness of this immobilization was confirmed from CV and EIS experiments. It was shown that immobilized Hb retained its bio-catalytic activity for Fe ions in Hb chains which was used to make a mediator-less H_2O_2 biosensor.

In the last step of the study, EFK8 and EFK8-SWNT hybrid hydrogels were prepared and used to culture NIH-3T3 fibroblast and A549 small cell lung cancer cells. The effect of the presence of SWNT in the peptide hydrogel on NIH-3T3 cells behavior cultured on hydrogels was first investigated. Inverted light and confocal microscopy images showed that although cells grow they tend to have spherical morphology and form colonies on the EFK8 hydrogel. The presence of SWNT significantly improved cell behavior so that they exhibited a stretched morphology, spread individually and homogeneously over the surface and proliferated faster. In addition, the cells were observed to migrate into the hydrogel after being seeded on top of the hydrogel. Micro-indentation tests showed that increasing EFK8 solution concentration led to an increase in the hydrogel compressive modulus, whereas presence of SWNT did not have any effect in this case. So the beneficial effect of SWNT on cell behavior cannot be attributed to mechanical property modification

and is probably due to the role of SWNT as locations for cell anchorage that facilitate cell attachment, spreading and migration. The cells encapsulated in both hydrogels showed the same behavior as in 2D (i.e., forming colonies on EFK8 and spreading individually on the hybrid hydrogel). In the second part of this study, the potential of EFK8 hydrogels for spheroid formation of cancer cells was explored. These cancer cell spheroids can be used as models for real tumors, to carry out drug screening in 3D cell cultures and to investigate the effect of the microenvironment on tumor progression and metastasis. It was observed that cells formed spheroids on EFK8 hydrogels at normal peptide concentrations, but exhibited more stretched morphology and migratory phenotype when seeded on the stiffer hydrogel. The cells also adopted a stretched morphology with higher migration when seeded on the EFK8-SWNT hydrogels. Again this behavior can be attributed to the facilitating effect of SWNTs for cell adhesion and migration. This effect can be used to study another effect of the microenvironment, namely cell-binding motifs, on tumor progression and metastasis.

Overall, by expanding the functionalities of both CNTs and self-assembling peptides, this work has introduced a new hybrid nanomaterial for bio-applications, especially biosensors, 3D cell cultures and tissue engineering.