Non-Thermal Plasmas in the Development of a MWCNT-Based Drug-Eluting Coating for Cardiovascular Metallic Implants

L. Hein[1], S. Coulombe[2] and R. Mongrain[1]

[1]*Department of Mechanical Engineering, McGill University, Montreal, Canada*
[2]*Department of Chemical Engineering, McGill University, Montreal, Canada*

Abstract: A multi-walled carbon nanotube-based coating was applied onto a pretreated 316L stainless steel plate by electrophoretic deposition. Good adhesion between the substrate and the coating was achieved by addition of a thin plasma polymer as an interlayer. Once deposited, the multi-walled carbon nanotube-based coating was functionalized using a low-pressure capacitively-coupled radiofrequency ammonia plasma. A maximum content of 15 at% nitrogen and 1.5% primary amine was reached.

Keywords: Non-Thermal Plasma, Multi-Walled Carbon Nanotubes, Drug-Eluting Coatings

1. Introduction

Ever since the introduction of metallic implants in blood-contacting environments, different strategies have been employed to improve their bio- and hemocompatibility with the aim of reducing the risk for short- and long-term complications. While conventional drug-eluting coatings are a promising alternative for this application, the development of new generation coatings is imperative and builds upon challenges faced by previous models.

Multi-walled carbon nanotubes (MWCNTs) have received a lot of attention as a drug carrier material due to their unique property pool. Requirements for efficient drug-delivery systems include a high drug loading capacity. MWCNTs can achieve this need due to their inner hollow space, and their extremely large surface area. Their high aspect ratio and mechanical strength, as well as their chemical stability and tuneability further qualify them as excellent vehicles for drug delivery [1]. MWCNTs can be grown on different metallic substrates by direct or catalytic chemical vapour deposition (CVD). CVD typically uses a hydrocarbon gas such as methane, acetylene or carbon monoxide as the carbon source and temperatures between 600 and 1200 °C [2]. The corrosion resistance of a permanent implant strongly influences its functionality, reliability, and biocompatibility. If metallic implant materials such as 316L stainless steel (SS) are exposed to high temperatures, their surface composition and morphology change, and their corrosion-resistant surface layers suffer partial or full degradation [3]. As such, they become highly susceptible to corrosion in physiological conditions and are no longer biocompatible. If MWCNTs are synthesized on 316L SS by CVD at high temperatures, the metallic substrate can no longer be used in permanent implant applications. Therefore, MWCNTs must be removed from the substrate they were initially grown on and deposited as a coating onto a pristine metallic surface.

A promising technique to coat bare metallic surfaces with MWCNTs in suspension is electrophoretic deposition. To ensure good adhesion between the MWCNTs and the pristine metallic substrate, the substrate surface must be pretreated by means of abrasion, wet chemical and/or plasma treatments. Thin plasma-synthesized interlayers can improve the adhesion between metallic surfaces and carbon coatings [4]. Removal of the MWCNTs from the surface they were synthesized on can be achieved by sonication in an appropriate solvent. The MWCNTs can then be charged with a surfactant to form a stable suspension which acts as the electrolyte. If a DC current is applied through the MWCNT suspension using two electrodes, the charged MWCNTs accumulate on the electrode of opposite charge. The determining factors for smooth MWCNT deposition are the stability of the MWCNT suspension, substrate pretreatment, applied potential, interelectrode spacing and treatment time. After electrophoretic deposition on a pristine metallic substrate, washing of the MWCNT coating with an organic solvent removes excess surfactant.

MWCNTs are inert and difficult to treat chemically. They are hydrophobic and agglomerate easily when dispersed in host fluids [1]. The two most widely employed approaches for tuning the chemical affinity and surface properties of MWCNTs are wet chemical and non-thermal plasma functionalization. Compared to most wet chemical processes, plasma functionalization has the advantage of shorter reaction times and is not accompanied by unwanted process waste and sample contamination, which is crucial in the preparation of nanomaterials for biomedical applications. Additionally, plasma processes offer a wide range of MWCNT functionalizations depending on the gas composition, power, treatment time, and pressure used. Regardless of the gases used, highly reactive species are formed in the plasma state which can initiate the formation of new covalent bonds after breaking the benzene rings of the MWCNT structure. Plasma treatment improves the reactivity of MWCNTs by introducing functional groups to individual tube walls. The introduction of polar groups converts the hydrophobic nature of pristine MWCNTs into a strong hydrophilicity required for in vivo applications. For the present investigation, the aim is to bind primary amine ($-NH_2$) groups to MWCNTs via radiofrequency plasma functionalization with ammonia. The focus lies on adding $-NH_2$ since they can be covalently coupled to carboxyl (-COOH) groups of various drug compounds via conventional cross-linking agents such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in combination with N-hydroxysuccinimide (NHS).

2. Experimental Method

MWCNTs were grown in-house following a thermal CVD batch process. They were synthesized on a 3.0 x 8.0 cm 316L SS mesh coupon (wire diameters: 0.07 and 0.05 mm, grid opening: 15 µm, thickness: 0.150 mm). The 316L SS coupon was first sonicated in fresh acetone for 40 min to remove any surface impurities or grease. After sonication, the coupon was left to air-dry and transferred immediately into a CVD tube furnace at 700 ºC with the loading chamber open to ambient air. After 2 min, the loading chamber door was shut, and argon was injected at a flow rate of 3000 sccm to lower the oxygen level inside the furnace below 0.5%. Argon flow was decreased to 592 sccm for 1 min before acetylene was co-injected at 68 sccm for 2 min. The acetylene flow was stopped while the argon flow continued for 2 min to give time for the MWCNTs to grow on the 316L SS coupon. Before retracting the MWCNT-covered 316L SS coupon, the furnace was purged with argon at 3000 sccm for 5 min and allowed to cool down to room temperature. The dimensions of the MWCNTs were found by means of a scanning electron microscope (SEM).

The as-produced MWCNTs were used for deposition on a pristine 316L SS plate (2.5 x 10 x 0.02 cm). The MWCNTs were first sonicated in an ice-cooled 0.2 g/L sodium dodecyl sulfate (SDS) aqueous solution at pH 10 for 60 min to remove them from the 316L SS mesh they were initially synthesized on. pH adjustments were made using a 10 M sodium hydroxide solution. The sonication led to the formation of a stable MWCNT-SDS suspension. The MWCNT concentration in the SDS solution was determined by weighing the SS mesh coupon that the MWCNTs were grown on before and after sonication, as well as by UV-visible spectroscopy of the MWCNT-SDS suspension. Prior to deposition, the SS plate was polished using 600 and 1600 grit paper, consecutively. The polished surfaces were soap-washed and air-dried before sonication in fresh acetone for 15 min. Then, a plasma polymer was applied to later serve as an interlayer for improved MWCNT coating adhesion. For this, the 316L SS plate was placed on the live electrode of a low-pressure continuous wave capacitively-coupled radiofrequency (13.56 MHz) plasma system (SS electrodes of 10 cm in diameter, separated by a 1.5 cm gap and the live electrode covered with a 3.5 mm-thick quartz disc). The reactor was flushed with argon at 2000 sccm for 3 min to evacuate residual air. The flow rates of argon and ethane, which served as the plasma polymer precursor gas, were set to 250 sccm and 1 sccm, respectively. Plasma polymer deposition was carried out for 30 min at 1 Torr and 20 W. Finally, the 316L SS plate was removed from the plasma chamber and immersed into the MWCNT-SDS electrolyte. A diagram of the setup is displayed in Fig. 1. To initiate the electrophoretic deposition, a constant DC voltage of 40 V was applied. The current variation over time was recorded. A graphite plate served as the cathode and the pretreated 316L SS plate as the anode. The interelectrode spacing was approximately 2 cm. The deposition was carried out until the initially black electrolyte became clear. Upon removal from the electrolyte, the MWCNT-coated 316L SS plate was gently rinsed with reverse osmosis water and immersed in acetone for 60 min to remove residual surfactant. The composition of the plasma polymer and the purity of the MWCNT coating were assessed by X-ray photoelectron spectroscopy (XPS) with an Al Kα source, micro-focused monochromator, and spot size of 200 µm. The morphology of the deposited MWCNT coating was examined using SEM and the coating roughness measured with a surface profilometer. The adhesion of the MWCNT coating to the SS substrate was characterized via scratch testing using a 20 µm diamond tip.

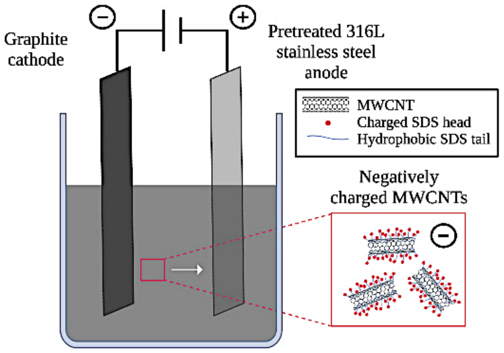

Fig. 1. Diagram of the electrophoretic deposition cell for MWCNT coating application

The MWCNT-coated 316L SS plate was then placed in the plasma reactor again, this time for plasma functionalization. The reactor was flushed with argon at 2000 sccm for 3 min to evacuate residual air. The argon flow was set to 250 sccm and prior to ammonia plasma functionalization, the MWCNTs were pretreated with an argon plasma at 30 W and 1 Torr for 10 min. To determine the experimental parameters which give rise to the highest degree of -NH_2 grafting by ammonia plasma functionalization, different RF power levels (5-200 W), treatment times (1-120 min), pressures (1-10 Torr) and plasma modes (continuous and pulsed) were investigated. For pulsed plasma treatments, various duty cycles (5-90 %) and pulse frequencies (100-10,000 Hz) were tested. The chemical composition of the pristine, argon plasma pretreated and ammonia plasma functionalized MWCNTs was assessed by XPS. The presence of -NH_2 was confirmed qualitatively by thermogravimetric analysis coupled with infrared spectroscopy (TGA-IR) in an argon/air atmosphere. Chemical derivatization by exposure to 4-trifluoromethyl benzaldehyde (TFBA) vapour for 5 hours was employed to quantify the amount of -NH_2 on the MWCNT surface.

3. Results and Discussion

The MWCNTs synthesized by CVD were 30-40 nm in diameter and 15-20 µm in length as confirmed by SEM. After removal of the MWCNTs from the synthesis substrate, their diameter remained unchanged, but their length decreased as the tubes broke off the SS mesh at any point along their length. The average MWCNT concentration in the SDS-MWCNT electrolyte was 0.078 g/L ± 0.024 g/L. During electrodeposition, negatively charged MWCNTs were effectively drawn towards the positively charged 316L SS plate. Since the electrolyte was water-based, hydrolysis of water took place. The oxygen formation at the pretreated SS anode did not interfere with the deposition of a smooth coating on the anode due to the inherent porosity of the MWCNT coatings. The chromium oxide surface layers of 316L SS anodes are typically prone to oxidization. In this case, the thin plasma polymer film which was applied on the 316L SS plate protected the chromium oxide against degradation. The surface composition of the plasma polymer prior to deposition was 81 at% carbon, 12 at% oxygen and 7 at% nitrogen. The presence of carbon can be explained by the use of ethane as a precursor gas while the presence of oxygen and nitrogen originate from residual air in the plasma chamber. Since the thickness of the plasma polymer layer was in the range of several nm, charges did accumulate on the surface of the SS anode. It further acted as an adhesive interlayer which the MWCNTs deposited on. After electrophoretic transport through the electrolyte, the MWCNTs agglomerated on the carbon-based plasma polymer film to form a coating. In addition to weak van der Waals forces, it is suggested that the adhesion between the plasma polymer and the MWCNTs is amplified by the formation of CH-π interactions.

While the applied voltage on the electrophoretic deposition cell was kept constant, the effective current decreased over time as more and more charged species were removed from the electrolyte and a growing MWCNT coating accumulated on the surface of the SS anode. The deposited MWCNT coating had a smooth and uniform black appearance. The MWCNT coating had a composition of 78 at% carbon, 19 at% oxygen and 3 at% sulfur prior to acetone washing. The sulfur content indicated the presence of residual SDS. After acetone washing, the carbon content of the MWCNT coating increased to > 90 at% carbon. The sulfur content decreased to near zero. The remaining oxygen impurities originate from partial oxidation of the MWCNTs during electrophoretic deposition. SEM analysis of the electrodeposited MWCNTs confirmed their integrity, heavy entanglement, and insignificant agglomeration as shown in Fig. 2. The average MWCNT coating thickness was 2.2 +/- 0.3 μm and the average surface roughness was 0.15 +/- 0.03 μm. Progressive scratch testing up to 10 N and over a length of 3 mm left behind a clean scratch. Along the entire scratch length, the coating remained intact and did not peel off the substrate (cf. Fig. 2). Additionally, the resulting friction force vs. scratch length graph did not show a spike in friction force which would have been characteristic of coating failure. These results indicate excellent coating adhesion.

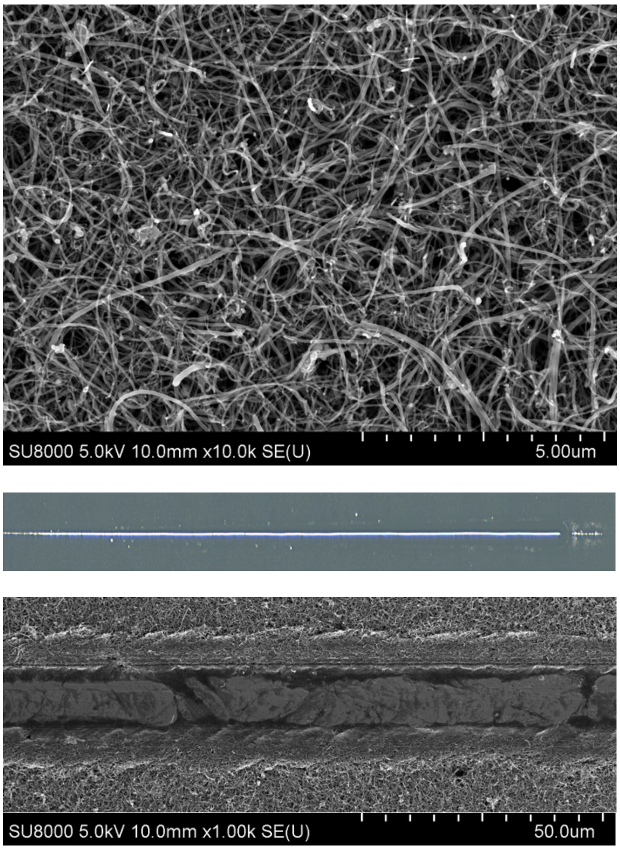

Fig. 2. (top) Morphology of the deposited MWCNT coating and scratch tested MWCNT coating at (middle) low and (bottom) high magnifications

The MWCNT coating was exposed to several continuous and pulsed radiofrequency plasmas in pure ammonia to bind $-NH_2$ onto the nanostructure. Prior to plasma functionalization, the MWCNTs were pretreated with argon plasma. The pretreatment rendered the MWCNTs more defective by ionic bombardment, which enhanced the effect of the following ammonia plasma treatment. Pristine MWCNTs contained no detectable amount of nitrogen. The best at% nitrogen results of 15 % were observed for continuous ammonia plasmas at 5 W, 1 Torr, a flow rate of 10 sccm, and a processing time of 45 min (cf. Fig. 3). For pulsed plasma treatments, an increasingly high at% nitrogen was obtained with an increasing pulsation frequency and longer duty cycles (cf. Fig. 5), which confirmed the appropriateness of using continuous plasmas. While three main types of nitrogen-containing groups ($-NH_2$, secondary amines (=NH) and nitriles ($\equiv$N)) can be chemically bonded to graphitic surfaces by ammonia plasma treatment, only the presence of $-NH_2$ is of interest and was evaluated qualitatively by TGA-IR and quantified by TFBA vapour chemical derivatization. The TGA-IR spectra revealed the presence of $-NH_2$ solely. Chemical derivatization by TFBA vapour showed an average percentage of 1.5% $-NH_2$ on the MWCNT surface for the most successful ammonia plasma functionalization protocol. As such, roughly 10% of the total at% nitrogen on the MWCNT surface were part of $-NH_2$ groups.

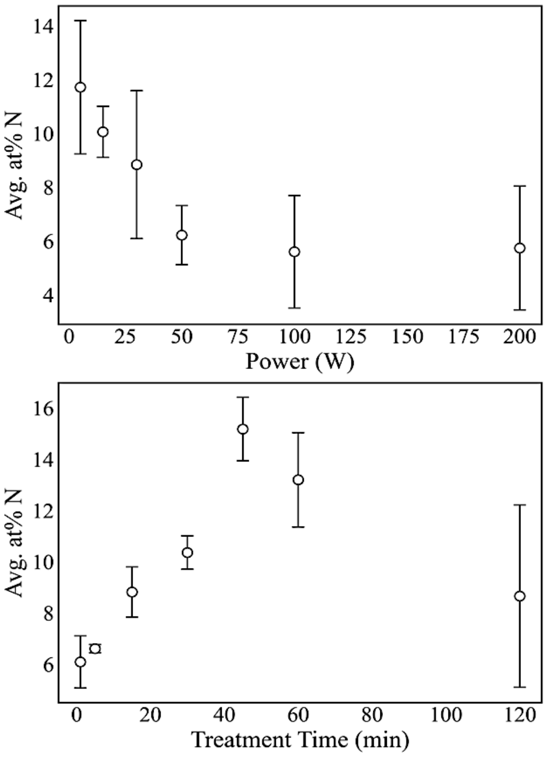

Fig. 3. Effects of (top) plasma power with other parameters constant (1 Torr, 10 sccm, 30 min) and (bottom) plasma treatment time with other parameters constant (1 Torr, 10 sccm, 15W)

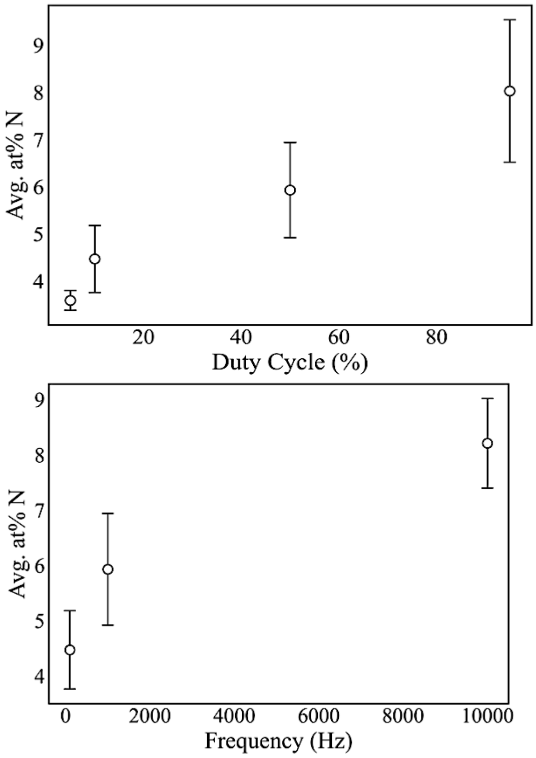

Fig. 6. Effects of (top) duty cycle with other parameters constant (15 W, 1 Torr, 10 sccm, 30 min, 1 kHz) and (bottom) pulsing frequency with other parameters constant (15 W, 1 Torr, 10 sccm, 30 min, 50 %)

4. Conclusion

In this study, we have successfully deposited a MWCNT coating on a 316L SS plate pre-coated with a thin ethane-based plasma polymer. The most successful combination of ammonia plasma functionalization parameters (continuous plasma, 5 W, 45 min, 1 Torr, 10 sccm) resulted in a maximum surface content of 15 at% nitrogen and 1.5% $-NH_2$. Through these preliminary efforts, it has been demonstrated that non-thermal radiofrequency plasma treatments, in the form of plasma polymerization and functionalization, are key enablers for a MWCNT drug-eluting coating technology. In the next phase of the study, we will investigate the drug loading and elution properties of the coating.

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