Prolonged delivery of siRNA from cathodic electrodeposited chitosan/siRNA complex on titania nanotube array

Yumei Zhang, Wen Song.

Department of Prosthetic Dentistry, School of Stomatology, Fourth Military Medical University, Xi'an 710032, China

Statement of Purpose: Titania nanotube array (TNT) represents a novel strategy for titanium surface topography modification. More recently, grafting biomolecules on TNT has attracted more attention to further biofunctionalize the TNT[1]. However, the RNA interference (RNAi) based approach to modify TNT hasn't been reported yet, although it has been immobilized on other titanium topography[2]. Traditional methods for delivery of siRNA from stent such as simple adsorption[3] or layer-by-layer[4] is less efficiency or time consumption. To seek a more effective and convenient way is essential. Cathodic electrodeposition (CED) of chitosan is a promising way to immobilize polymer on conductor surface, which requires short process time and simple apparatus without other agents[5]. Meantime, chitosan is also an excellent siRNA carrier that could protect and deliver siRNA into cells. Therefore, it might be possible to incorporate siRNA and chitosan onto TNT surface simultaneously via CED. Our study focus on this idea and has made some advances.

Methods: Pure titanium foil (99.8% purity, 5×5×2mm^3) was polished to 2000 grid and the TNT was anodized in 0.5wt% hydrofluoric acid at 20V for 30min by using a DC power supply. Chitosan(Mw=100~300 kDa, DD=93.37%, MP Biomedicals) was dissolved in 0.04M HCl overnight at the concentration of 2mg/ml and the pH was adjusted to 5 by 1M NaOH. To prepare the CED electrolyte, 100µl siRNA(20µM, target TNF, gift from iNANO) and 1000µl chitosan solution were vortexed vigorously for 1min. The distance between electrodes was 1cm and the CED duration was 3min. Several voltages (1.5, 3.5, 5V) were applied and the deposited coatings were observed and quantified by Scanning Electron Microscope (SEM, S-4800) and RiboGreen (Invitrogen) separately. Water contact angle was also measured. To further visualize the coating, FITC-labeled chitosan (Refer to Huang M et al.[6]) and Cy3-labeled siRNA (target EGFP, gift from iNANO) were used in the system and 5V voltage was applied. Fluorescence images were taken by inverted fluorescence microscope (OLYMPUS). To figure out the degradation profile of the CED coating, samples (5V) were immersed in PBS buffer at pH of 7 and 5. At selected time points, the PBS was collected and replace with fresh. After quantification by RiboGreen, the residual siRNA on TNT was calculated manually. Several SEM images were also taken after incubated for certain period.

Results: Chitosan was grafted on the walls of TNT and the entrance of nanotube was covered with more and more polymer until no nanotube structure could be seen when the voltage increased (Fig.1A). The incorporated siRNA also augmented linearly from ~0.2µg to ~0.6µg (Fig.1B). Similarly, water contact angle was linearly elevated from ~25^o to ~50^o due to the increase coverage of hydrophobic chitosan (Fig.1C). Consequently, 5V CED voltage was optimized in order to acquire maximum siRNA deposition. Fig.2 revealed that the siRNA (red) and chitosan (green) were integrated well on TNT surface, which implied that the chitosan/siRNA complex was stable during the CED procedure. The rigid combination of chitosan and siRNA was critical to protect and deliver siRNA into cells.

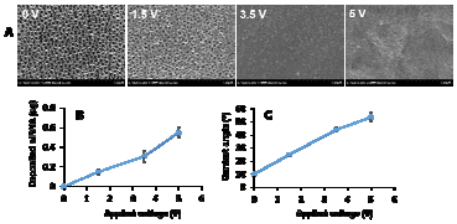

Figure 1. (A) SEM images (×50k) of CED surface; (B) siRNA quantification; (C) Contact angle.

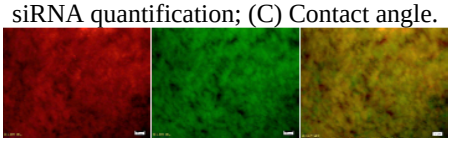

Figure 2. Fluorescence images (×20) of coating.

The coating degraded rapidly within one week once immersed in acid circumstance (Fig.3, pH=5). However, in neutral condition, the coating could retain siRNA for more than two weeks (Fig.3, pH=7).

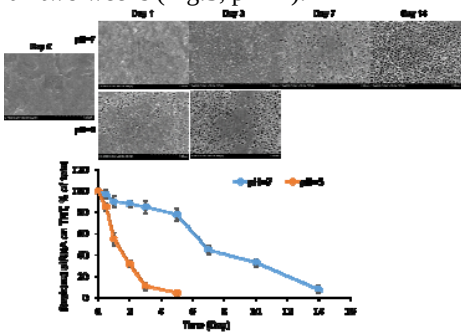

Figure 3. Degradation profile of CED coating.

Conclusions: Incorporation of siRNA and titanium is a tough problem because of the vulnerable siRNA that results in difficulty of immobilization and long term maintenance. Our study provided a convenient strategy based on the CED technique to deposit chitosan/siRNA on TNT surface. The deposited coating could maintain siRNA for over two weeks in neutral condition, which was pivotal to functionalize titanium in a prolonged fashion. The further study will concentrate on the cellular effect of the CED coating. Our method not only establishes the organized chitosan/siRNA coating on TNT, but can also be used in other conductors and charge polymers, for different purposes.

References: [1] Lai M, Biomacromolecules 2011;12:1097-105.[2] Song W, RSC Advances. 2013;3:11292-300.[3] Wu K, ACS applied materials & interfaces. 2013;5:2733-44.[4] Hossfeld S, Acta biomaterialia. 2013;9:6741-52.[5] Jiang T, Biomacromolecules. 2010;11:1254-60.[6] Huang M, Pharm Res. 2002;19:1488-94.