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Formation of Polyethylenimine/Carrageenan Bi-layer as Monitored by Atomic Force Microscopy and Biomolecular Interaction Analysis

Annabelle V. Briones[1] and Toshinori Sato[2]

[1]Industrial Technology Development Institute
DOST Compd., Gen. Santos Ave., Bicutan, Taguig City, Philippines
[2]Dept., of Biosciences and Informatics, Keio University
3-14-1 Hiyoshi, Kouhoku-ku, Yokohama, 223-8522, Japan

Abstract Atomic force microscopy (AFM) was used to monitor the formation of polyethylenimine (PEI)/carrageenan bi-layer for potential use as anti-bacterial coating on biomaterial surfaces. All samples were prepared in phosphate buffer solution and applied to mica disk alternately. The micrographs showed the formation of bi-layer of polyethylenimine and carrageenan (κ, ι, λ) as observed in the change of height of the layer and surface morphology. The bimolecular binding of carrageenan with polyethylenimine was also investigated using a biosensor. The sensorgram showed that PEI interacted molecularly with carrageenan. Results were: 1,916.08 pg/nm^2 for *κappa* type; 1,844.1 pg/nm^2 for *ιota* type and 6,074.24 pg/nm^2 for *lambda* type.

Keywords: Atomic force microscopy, Polyethylenimine, Carrageenan

1. Introduction

The use of layer-by-layer technique as anti-bacterial coating on biomaterial surfaces is becoming popular. This technique is based on alternating deposition of oppositely charged synthetic or natural polyelectrolytes to form a thin film layer-by-layer assembly [1,2]. Previous studies done using this technique was the use of chitosan/κ-carrageenan multi-layers [3]. In this study, polyethylenimine was used instead of chitosan. It was then layered with the three types of carrageenan. Atomic force microscopy imaging was done to determine the morphology of bi-layers.

2. Materials & Methods

2.1. Chemicals

PEI (average MW=25KDA, branched with degree of polymerization of 580) was obtained from Aldrich Company (St Quentin Fallavier, France). κ-carrageenan (Bengel KK-100, Lot No. XO300-2), ι-carrageenan (Benvisco SI-100, Lot No.M1400-1) and λ-carrageenan(Benvisco SL-100, Lot No. S2703-2) was obtained from Shemberg Biotech Corporation (Carmen, Cebu, Philippines, 6005). Dulbecco's PBS (-) was obtained from Nissui Pharmaceutical Co. Ltd (Japan).

2.2. Sample preparation

κ, ι, λ carrageenan and PEI were dissolved in PBS (-) solution then mixed using a vortex machine, followed by ultrasonication and shaken at 200 rpm for 8 hours. Final concentration of each sample is: 1 mg/mL.

2.3. Preparation of sample for AFM analysis

The silica glass was dipped in carrageenan solution then allowed to dry at room temperature followed by washing with water to remove excess buffer solution and allowed to dry again. The dried coated silica glass was immersed in PEI solution, dried and washed with water followed by final drying at room temperature.

2.4. Atomic force microscopy (AFM) measurement

Imaging was done through a commercial SPA-300 system of Seiko Instruments, Inc. Japan. A Si_3N_4 tip on the cantilever with a length of 100μm and a depth of 400 nm (SN-AF01-A, Olympus Optical Co.) was used with tapping mode. Imaging was recorded in non-contact mode under ambient condition. Solutions of PEI and carrageenan on the mica disk were observed as control.

2.5. Biomolecular interaction analysis

Analysis of biomolecular binding of carrageenan and PEI was done using BIACORE X, Pharmacia Biosensor AB, Uppsala, Sweden equipped with sensor chip C1 BIACORE Lot no. 10020781. Samples of κ, ι, λ-carrageenan solution and PEI solution at 1 mg/mL concentration was injected at 120 uL with a flowrate of 10 uL/min.

3. Results and Discussion

Morphologies of κ, ι, λ type carrageenan are shown in Fig. 1. κ-carrageenan (Fig. 1A) forms an aggregated network in conformity with the results of McIntire and Brant, 1999 [4] who uses water to dilute the carrageenan samples and prepared by aerosol spray deposition. Polymer chains are separated with some overlap. The network is composed of a continuous bifurcated fibrous structure [5]. ι-carrageenan forms a compact network as compared with κ-carrageenan. As seen in Figure 1C, there was no separation of polymer chains since the molecular structure of ι-carrageenan has "kinks" that increase its flexibility and reduce the space occupied by the molecule [5], this might explain the irregularities of the structure. It forms a homogeneous and continuous polymer network. In λ-carrageenan (Fig.1E) the structure exhibited a network or typical chains for the polymeric molecule. It has the same morphology as described by Oliva *et al*, 2003 [6] in their studies. PEI film (Fig. 1G) shows a network that is similar to κ-carrageenan but its polymer chains are arranged uniformly.

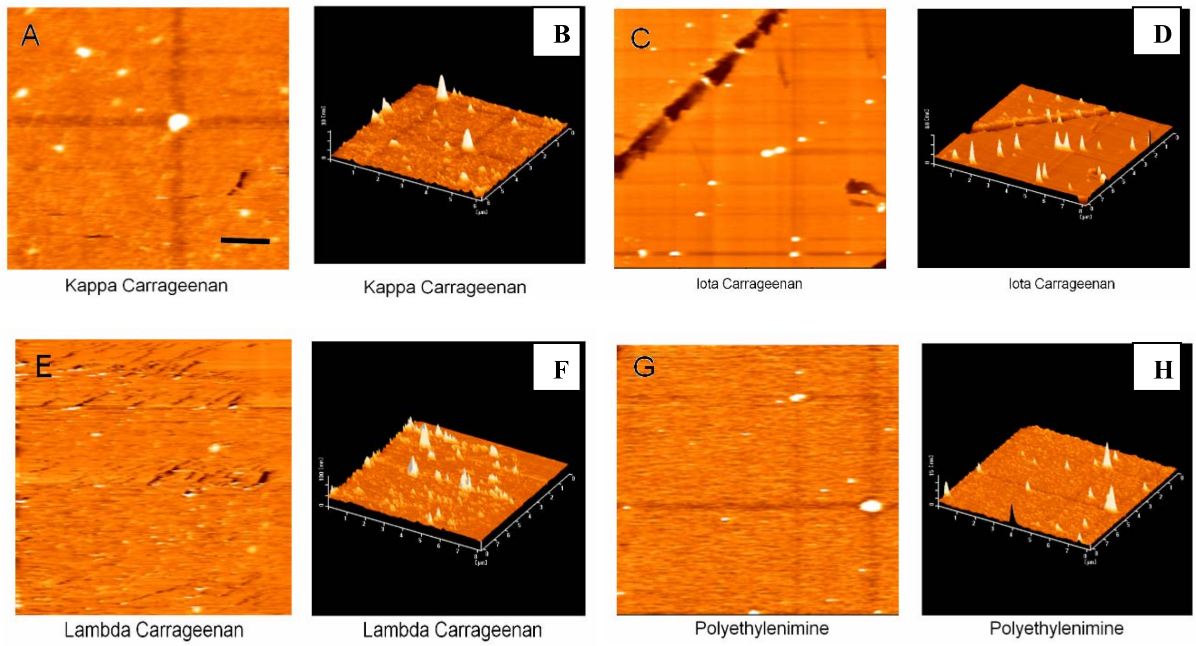

Figure 1. AFM micrographs of κ, ι, λ carrageenan and polyethylenimine. Scale bar: 2 um.

Figure 2 shows the AFM micrographs of the PEI/carrageenan bi-layers. The images (Fig.2 A,C,E) shows the presence of nanorings confirming the formation of layer -by- layer assembly. The change in height of the bi-layer film was observed in the images (Fig. 2B,D,F) as compared to the images of single polymer (Fig. 1 B, D, F,H). Table 1 shows the change in height of the single polymer layer and bi-layers.

The formation of bi-layer was further analyzed using a biosensor (BIACORE X). Figure 3 shows the sensorgram of the interaction between different types of carrageenan and PEI. The binding capacity of PEI with carrageenan is tabulated in Table 2. Results showed that PEI interacted molecularly with carrageenan.

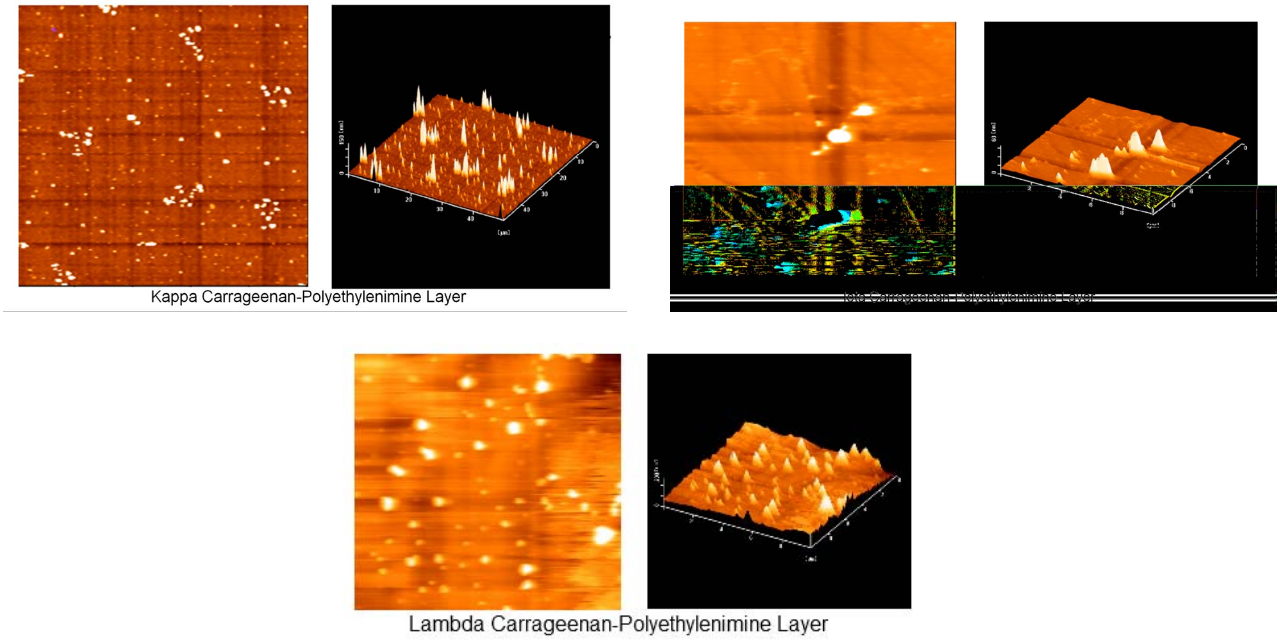

Figure 2. AFM micrographs of PEI/carrageenan layer.

Table 1. Height of single polymer and bi-layer of PEI and carrageenan

	Height (nm)
Kappa carrageenan	30
Iota carrageenan	60
Lambda carrageenan	100
Polyethylenimine (PEI)	15
Kappa carrageenan/PEI bi-layer	150
Iota carrageenan/PEI bi-layer	60
Lambda carrageenan/PEI bi-layer	200

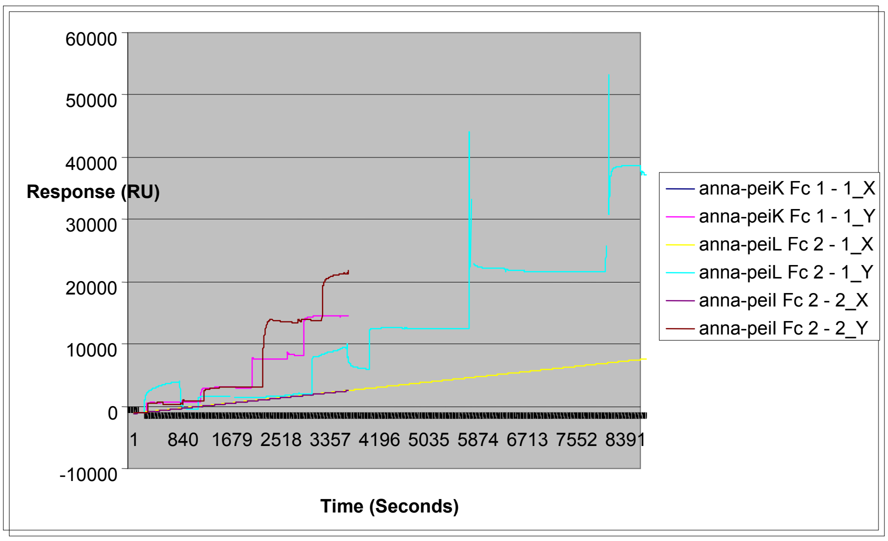

Figure 3. Sensorgram of the interaction of PEI and different types of carrageenan.

Table 2. Binding capacity of PEI with different types of carrageenan

	Binding capacity (pg/nm^2)
Kappa carrageenan	1,916.08
Iota carrageenan	1,844.1
Lambda carrageenan	6,074.24

3. Summary and Conclusion

The study showed that through AFM imaging and biosensor analysis, bi-layer formation of PEI and carrageenan can be monitored and evaluated.

4. Acknowledgment

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5. References

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