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硕　士　学　位　论　文

材料表面电荷对大肠杆菌粘附行为影响的研究

The Study of Effects of Surface Charge on the Adhesion Behavior of *Escherichia coli*

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摘 要

相比于浮游生存方式，细菌更倾向于粘附在材料表面生存。细菌粘附在材料表面最终会导致生物膜的形成，对生物医疗设备、水存储及处理系统、海洋船舶和食品等研究和应用领域造成严重危害。细菌在固体表面的粘附过程十分复杂，主要受细菌自身特性、材料表面性质及环境特性等因素的影响。细菌在水溶液中通常呈电负性，因此，深入了解细菌与不同带电表面的相互作用对开发抗菌材料具有重大意义。

由于氧化铟锡（Indium-Tin-Oxide，ITO）玻璃具有良好的物理性质，如在生理条件下的稳定性，良好的透明性和导电性等特点，因此 ITO 非常适用于研究材料表面改性对细菌粘附的影响。在本文中，我们以 ITO 导电玻璃为基底，制备了磺酸基团修饰的带负电的表面和季铵基团修饰的带正电的表面。ITO 表面首先经氧气等离子体预处理，使得 ITO 表面羟基化，然后分别用硅烷偶联剂（3-巯丙基三甲氧基硅烷和 3-氯丙基三甲氧基硅烷）处理，再分别经过氧化反应和季胺化反应制备出带负电和正电的表面。修饰前后的 ITO 表面用静态水接触角（WCA）、原子力显微镜（AFM）、循环伏安法（CV）、交流阻抗法（EIS）、X-射线光电子能谱（XPS）、甲苯胺蓝（TB）和荧光素（钠盐）吸附试验进行表征。本文利用革兰氏阴性菌-大肠杆菌（*E. coli* K-12）作为测试菌株来评价修饰材料表面的抗菌性能及机理。平板涂布法、激光共聚焦显微镜（CLSM）和扫描电子显微镜（SEM）等方法用来检测材料表面的抗菌性能以及吸附在材料表面的大肠杆菌的活性和形态，从而为细菌与带电表面之间的相互作用提供详细信息。本论文主要得到以下结论：

（1）WCA 和 AFM 测试表明 ITO 表面修饰前后的接触角和粗糙度均有一定程度的变化，但总的来说，对本实验中的细菌粘附影响不大。因为修饰前后 ITO 表面都是相对亲水的，且 *E. coli* 的尺寸比表面粗糙度的值大三个数量级。

（2）CV 和 EIS 测试表明，硅烷偶联剂已经成功自组装到 ITO 表面，从而阻碍了电极与溶液中氧化还原物质间的电子传递。而经过氧化或者季胺化反应后，ITO 表面又恢复了一定的电化学活性，表明 ITO 表面已成功修饰上磺酸基团和季

铵基团。通过 XPS 对特征元素 S、Cl 和 N 等的检测，进一步表明 ITO 表面已成功修饰上目标官能团。

（3）用 TB 对磺酸化表面的磺酸基团进行标定，结果说明 ITO 表面已成功磺酸化。用荧光素（钠盐）对季胺化表面的季胺基团进行标定，结果说明 ITO 表面已成功季胺化，且季胺化程度较高。

（4）平板涂布结果表明带负电荷的表面和带正电荷的表面均能抑制细菌初始阶段的粘附，但是带负电的表面抗菌效果更好。CLSM 结果表明，带负电荷的材料表面并没有杀死细菌而带正电荷的表面则使细菌失活。SEM 结果表明，大肠杆菌能够产生鞭毛等细胞器从而吸附在空白 ITO 表面，而带电表面粘附的细菌并没有鞭毛的产生，这表明，鞭毛参与细菌的粘附过程，有助于材料表面上的生物粘附以及生物膜的形成。

关键词：表面电荷，粘附，大肠杆菌，鞭毛，ITO

Abstract

Bacteria prefer attaching to solid substrates rather than dwelling in a planktonic state, eventually leading to the formation of biofilms and surface-bound communities of microbes may cause adverse consequences in the research and application fields, e.g., biomedical devices, water storage and treatment systems, marine vessels and the food industry. The adhesion of bacteria onto solid surfaces is a complicated process that is influenced by various factors, including bacterial properties, material surface properties and environmental factors. Bacteria typically carry net negative charges in aqueous suspension; therefore, a deeper understanding how bacterial interact on different charged surfaces is very important for the further development of anti-biofouling surfaces.

Due to its attractive physical properties, such as stability under physiological conditions, special transparent and conductive properties, the indium tin oxide (ITO) surface is suitable for studying the influence of surface modifications on bacterial adhesion. In this paper, we modify the ITO surfaces with sulfonic groups (negatively charged) and quaternary ammonium groups (positively charged). The ITO surfaces were fabricated via oxygen plasma pretreatment, followed by treatment with silane-coupling agents ((3-mercaptopropyl) trimethoxysilane or (3-chloropropyl) trimethoxysilane). After the oxidation or quaternization treatment, a negatively charged surface and a positively charged surface were prepared. The modified surfaces were characterized by water contact angle, atomic force microscope, cyclic voltammetry, electrochemical impedance spectroscopy, X-ray photoelectron spectroscopy, TB colorimetric method and fluorescein sodium salt assay. In this study, Gram-negative *Escherichia coli* (*E. coli* K-12) cells were used to evaluate the antibacterial adhesion characteristics of the ITO surfaces. The spread plate method, confocal laser scanning microscopy (CLSM) and scanning electron microscope (SEM) were used to detect changes in the viability, morphology and structure of

Gram-negative *E. coli*, which provided detailed information for understanding the interaction between the charged surfaces and bacteria. The main conclusions of this study are listed as follows:

(1) The WCA and AFM measurements revealed that the wettability and roughness of ITO surfaces changed slightly after modification. However, the effect of wettability and surface roughness on the bacterial adhesion could be neglected in the present study because the bare and modified ITO surfaces were hydrophilic in general, and the size of *E. coli* was three order of magnitudes larger than the roughness values.

(2) CVs and EISs showed that silane-coupling agents were successfully grafted onto bare ITO surfaces and acted as barriers for the electron transfer between the electrode and redox species in the solution. After the oxidation or quaternization reaction, ITO electrodes showed electrochemical response again, indicating that ITO surfaces had been successfully modified with sulfonic acid groups and quaternary ammonium groups. Through the detection of the specific elements, such as S, N and Cl, the XPS experiments further confirmed that the ITO surfaces had been modified by the target functional groups.

(3) The TB colorimetric method allowed the quantification of the sulfonic acid groups on the ITO surface, indicating that the ITO surface had been successfully sulfonated. The cationic charge density of the modified glass slide was determined using fluorescein (sodium salt) staining. The results showed that ITO surface was successfully quaternized with a high degree of quaternization.

(4) The viable bacterial count test showed that both negatively and positively charged surfaces can reduce the initial bacterial adhesion, and surfaces with negative charges showed more inhibitory effects on bacterial adhesion. CLSM suggested that the negatively charged surfaces did not ‘kill’ the bacteria but that the positively charged surface did. The SEM images showed that the *E. coli* produced flagella on the bare ITO surface and no obvious flagella were observed on the charged surfaces, indicating that flagella are involved in bacterial attachment process and will help to control bioadhesion and biofilm at interfaces.

Keywords: Surface charge, Adhesion, *E. coli*, Flagella, Indium tin oxide

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