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Interaction force measurements for the design of tissue adhesives

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Abstract

Synthesis of tissue adhesives had been carried out in various laboratories in the past decades but the development is currently stalled. One of the key reasons, it is believed, is that researchers have not fully understood and resolved the role of the functional groups that are responsible for good adhesion to biological tissues. Further progress in synthesis is significantly hindered without this fundamental understanding. With this aim in mind, atomic force microscopy (AFM) has been exploited in this work to study the interactions between functional groups that are common to biological tissues. In this work, the AFM tip and substrates were functionalized and used to measure the non-specific interaction among these common functional groups. The ultimate aim of the study is to calculate the interaction force between a single pair of functional groups. A novel calculation method based on the AFM data and probe geometry is presented. The results provide insights into the strength of the bond between different functional groups and the could serve as a guide in selecting the appropriate functional groups in tissue adhesive synthesis. This method could be further applied to studies involving interfaces of biomedical devices where intermolecular interactions are of concern.

Keywords: Tissue adhesive; Interaction force; Functional groups; Atomic force microscopy

1. Introduction

Research in tissue adhesives has attracted attention in the biomedical field due to its potential handiness and ease of use. A good tissue adhesive that can harden within a few seconds to 1–2 min would be ideal for surgeons to shorten their operation time and would substantially eliminate the need (and also the fear of patients) for subsequent procedures for suture/staple removal. It can also be applied to some constrained regions, to which suture is not accessible. Biodegradability of the polymeric glues should make it a good temporary grip for severed wounds and lacerations since it would degrade gradually at a pre-designed rate, transferring the load back to newly grown tissues.

The practicality is, however, not as simple as the concept itself. This is because the physiological environment is too complicated for good adhesion to form and no one single solution can suit all the crucial requirements, including biocompatibility, biodegradability, strong bonding under wet conditions, non-toxicity of degradation by-products, rate of hardening, strength and flexibility, non-thrombogenicity, etc. These factors make the development of tissue adhesive systems a difficult task.

Currently, there are only two main types of commercialized tissue adhesive products, cyanoacrylate adhesives and fibrin adhesives, which are of synthetic and natural origin, respectively. These are the most extensively used tissue adhesives and are used in different surgeries. Cyanoacrylate glues have been used in facial plastic surgery, scalp wounds, otorhinolaryngology and childhood lacerations, and are considered an alternative to suturing [1]. Fibrin adhesives are even more widely used because of their biocompatibility. Fibrin adhesives have been used in cerebral surgery [2], skin grafts in burn injuries [3], cardiovascular surgery [4,5], nerve cell reconnection and others [6]. However, there are limitations that restrict the use of these

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adhesives: cyanoacrylate is not biodegradable and it releases formaldehyde upon degradation [7]; and the strength of fibrin adhesive is rather weak for load-carrying and flexible body parts [8]. Furthermore, these glues are very expensive to produce, and the high prices of the products cannot be afforded by most patients. This is especially true for fibrin adhesives.

New and novel tissue adhesive systems are definitely needed and this has provoked interest in the research field. In the past two decades, due to the increasingly promising applications of polymers in biomaterials, researchers have started to put their effort into synthesizing tissue adhesives. The syntheses include the modifications of biocompatible and/or biodegradable polymers such as urethanes [9,10], lactide/caprolactone [11,12], PEG hydrogel [13–15], gelatin [16–21] and chitosan [22–24]. In general, there are two approaches: grafting functional groups which are believed to have adhesive properties onto the main polymer chain; and mimicking nature's adhesion mechanisms. In the second approach, proteins involved in natural adhesives produced by mussels [25] and barnacles [26] were studied and synthesized. Some of these novel adhesives may seem to work from a macroscopic point of view but have been little studied microscopically to obtain a better understanding of the origin of the adhesiveness of these glues.

Atomic force microscopy (AFM), a scanning probe microscope that works at nanoscale resolution, is a powerful tool for studying the interaction forces between two contacting surfaces or even molecules. The operating principle of AFM is by "feeling" the force exerted onto the probe by the topological features of the substrate. AFM was extensively used in biomolecular study on the strength of interaction of ligand–receptor [27,28], antibody–antigen [29], complementary DNA strands [30,31], and the molecular mechanical properties of single molecules [32–34]. In the specific studies of intermolecular interactions, probe and substrate are coated or functionalized with desired chemicals and the interaction of these pairs of chemicals on the substrate and probe were studied. Frisbie et al. [35] reported the force–extension curves of all combinations between hydrophilic COOH and hydrophobic CH_3 surfaces. They then ranked the strengths of tip–surface interactions in ethanol in descending order: COOH/COOH → CH_3/CH_3 → COOH/CH_3. vanderVegte et al. [36] functionalized the probe and surface with CH_3, OH, NH_2, COOH and $CONH_2$ using ω-functional *n*-alkanethiol and measured the interaction in ethanol. The authors believed that the adhesion force arising from these combinations (except CH_3) is actually due to the formation of hydrogen bond since the bond strength can vary depending on molecule polarity, bond length, environment, etc. The dependency of the adhesion force on pH value was also reported [37,38]. Furthermore, measurement of secondary interactions was applied in imaging the surface with different patches of functional groups. Detailed review papers on the development of this so-called "chemical force microscopy" (CFM) have been published [39,40].

In this work, AFM was used to measure the interaction forces of the functional groups that are common in biological tissues with the aim of shedding some light on the groups responsible for good adhesion. Secondary interactions were the focus because the chance of primary bond formation between adhesives and tissues is slim, due to the fact that these chemical reactions require external energy and/or catalysts which are usually toxic. In our AFM studies, the probe and substrate were functionalized with simple functional groups and the interactions between these surfaces in air were observed in the collected retraction force curves. Analyses of the force curves (in particular, the interaction force and the maximum deflection of the probe) and the probe geometrical factors led to a novel mathematical model for the calculation of interaction forces of a single pairs of interacting functional groups. Calculations of single–pair interaction force could serve as a guide to a better design of future tissues adhesives.

2. Method and equipment

2.1. AFM probe

The probe used was Pointprobe® LFM Probe supplied by Nanoworld, Switzerland. It is a soft cantilever that has a small spring constant, i.e. nominal value = 0.2 N m^{-1} (range from 0.02 to 0.7 N m^{-1}). The advantage of soft cantilever is that it strongly deflects when a given force is encountered. The cantilever beam is made of highly doped silicon with a nominal dimension of 225 μm × 48 μm × 1 μm, a sharp pyramidal tip of height ~10–15 μm and a radius of curvature of less than 10 nm. Its nominal resonance frequency is ~23 kHz. The detector side of the cantilever is coated with a 10 nm thick aluminum layer to improve its reflectivity to laser beam by a factor of 2.5.

The probes were functionalized with different functionalities using a self-assembled monolayer (SAM) technique. The procedure is elaborated in the following section.

2.2. Functionalization of probes and preparation of substrates

The functionalized surfaces were prepared in two different ways. To functionalize the probes and the surfaces with acid (COOH) and hydroxyl (OH) groups, we used the alkanethiolate–gold SAM method. First, the probes and the silicon surfaces were cleaned by a regular four-step cleaning procedure. In brief, they were washed in chloroform for 10 min and then immersed in piranha (3:1 v/v of sulfuric acid and 30% H_2O_2 in water) solution for 30 min. They were washed with a copious amount of deionized water before they were put into a 180 °C vacuum oven to dry the surface completely. After the cleaning steps, the surfaces of the probes and the silicon were coated with a layer of gold with thickness between 50 and 100 nm in the vacuum evaporator (JOEL JEE-420 Vacuum Evaporator) under a high vacuum of less than 4×10^{-4} Pa. The

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