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Attachment of micro- and nano-particles on tipless cantilevers for colloidal probe microscopy

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ABSTRACT

Hypothesis: Current colloidal probe preparation techniques face several challenges in the production of functional probes using particles ⩽5 μm. Challenges include: glue encapsulated particles, glue altered particle properties, improper particle or agglomerate attachment, and lengthy procedures. We present a method to rapidly and reproducibly produce functional micro and nano-colloidal probes.
Experimental: Using a six-step procedure, cantilevers mounted on a custom designed 45° holder were used to approach and obtain a minimal amount of epoxy resin (viscosity of ~14,000 cP) followed by a single micron/nano particle on the apex of a tipless cantilever. The epoxy and particles were prepared on individual glass slides and subsequently affixed to a 10× or 40× optical microscope lens using another custom designed holder. Scanning electron microscopy and comparative glue–colloidal probe measurements were used to confirm colloidal probe functionality.
Findings: The method presented allowed rapid and reproducible production of functional colloidal probes (80% success). Single nano-particles were prominently affixed to the apex of the cantilever, unaffected by the epoxy. Nano-colloidal probes were used to conduct topographical, instantaneous force, and adhesive force mapping measurements in dry and liquid media conveying their versatility and functionality in studying nano-colloidal systems.

1. Introduction

The atomic force microscope (AFM), developed by Binnig et al. [1] upon the principles of the scanning tunneling microscope and stylus profilometer, has become an essential technique for imaging the topography of surfaces at an atomic level and obtaining force-vs.-distance measurements in both air and liquid mediums [2]. Colloidal probe microscopy is a powerful AFM tool [3] which uses a cantilever with a spherical particle attached at the apex – instead of a traditional sharp pyramidal or conical tip – to study nano-scale forces between individual colloid particles. Furthermore, the colloidal probe technique can also be used to study the tangential forces present between particles and the elasticity of materials [4–10]. Particle interactions are critical in understanding the fundamental forces that are present in several colloidal systems including paints, paper, soil, clays, pharmaceutical drug particles, food applications and (in some circumstances) cells [11–15]. The forces measured through colloidal probe microscopy can provide insight into binding energies, adhesive properties, and in pharmaceuticals can also be used to understand formulation stability and drug particle interactions.

Prefabricated colloidal probes using gold, borosilicate glass, silicon dioxide and polystyrene spheres are commercially available; however, it is more applicable to use probes produced from materials present in the studied colloidal system. In principle, any material glued to an AFM cantilever can be used; however material in the form of spherical particles are preferred for practical reasons and easy comparison to theoretical models, such as the JKR [16] and DMT [17] models [18]. To study actual particle and particle-material forces present within a specific colloidal system of choice, one must be able to properly attach a colloid particle to the apex of a cantilever.

Previously published colloidal preparation methods proven successful for attachment of large micron particles (>10 μm) include using: commercial micromanipulators to obtain glue (Loctite) and silica spheres on a cantilever [19]; thin copper wires (~40 μm diameter) to position both the glue (Epikote 1004) and silica particle near the apex of the cantilever under an optical microscope [20]; and physically sintering silica or glass beads into a cantilever [21,22].

Abbreviations: CPM, colloidal probe microscopy; AFM, atomic force microscopy; MDI, Metered Dose Inhalers.

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These techniques are not transferrable with ⩽5 μm particles especially of pharmaceutical or biological value due to the inability to physically sinter and accurately manipulate such particles using 40 μm sized wires.

Other published methods that are capable of attaching smaller particles (~5–10 μm) – although with several limitations – include using the AFM [23,24] or using an angled cantilever holder in conjunction with an optical microscope [25] to manipulate cantilevers during the process. AFM methods require the use of multiple cantilevers to prepare a single probe. These methods are characterized by long preparation times due to cantilever alignment procedures and result in the attachment of multiple particles due to the inability to locate a particle of interest without completing an AFM scan. Manipulation using an angled cantilever holder is currently the most functional method used by several others pursuing colloidal probe microscopy in pharmaceutical and biological applications [26–29]. This method, developed by Preuss and Butt in 1998, requires attachment of a cantilever on a 30° angle holder to approach epoxy (Epikote 1004 Shell) and particles affixed to a glass slide in the focal plane of an optical microscope. To be workable, the epoxy must be heated above 50 °C using an aluminum strip adhered onto a cover slide. Common and significant difficulties faced when attempting to attach ⩽3 μm sized particles using this and previous methods discussed include:

1 Complete particle encapsulation by the glue.
2 Difficulty in reproducibly attaching a particle at the apex of the cantilever to maximize functionality.
3 Difficulty to attach a single particle.
4 The requirement to heat the stage, which may induce changes in material properties.

Here we report a method, evaluating key material set up and technique parameters, to address these limitations and reproducibly prepare submicron and nano-colloidal probes that can be used to evaluate pharmaceutical and biological submicron-colloidal systems in dry and liquid media.

2. Experimental section

2.1. Materials

Porous phospholipid-based particles, used to prepare all colloidal probes and sample substrates, were produced via a previously published spray-drying method of an emulsion formulation (containing diastearoyl-phosphatidylcholine, calcium chloride, perflooctyl bromide, and deionized water) [30,31]. Particles were sized using a Sympatec HELOS (Clausthal-Zellerfeld, Germany) equipped with an R1 lens and Aspiros unit and ranged from submicron to 5 μm, with an optical mean diameter of 2 μm. Veeco Model NP-O10 (levers A–D) tipless probes (Veeco, Plainview, NY, USA) and Hardman Double-Bubble Epoxies (Royal Adhesives & Sealants, Wilmington, CA, USA), two-component structural adhesives provided generously in the form of sample sachets, were used in colloidal probe preparation. During method optimization several Hardman epoxies varying in viscosities were evaluated including Double-Bubble products: BLACK regular setting (3.700 cPs), GREEN water-clear transparent (14,000 cPs), and RED fast setting (40,000 cPs). Viscosity values were provided as material properties by the manufacturer (Hardman). Previously used epoxies (Epikote 1004 and Epotek 377) were not used in this study as a comparison since they required heating of the stage for application, which can result in physical or chemical alterations to the particles during probe preparation. An Olympus CX41 optical microscope with 10× and 40× objectives was used to manipulate and load colloidal probes.

Custom designed cantilever and glass slide holders (Fig. 1) were developed using AutoCAD software (Autodesk Inventor Professional, San Francisco CA, USA) and produced with a Stratasys 3-Dimensional Elite Printer (Tasman Machinery, Cheltenham, VIC, Australia). All holders were made of an ABS thermoplastic polymer (P430). The cantilever holder was attached to a stock microscope slide for easy manipulation (Fig. 1).

2.2. Production of glass slide with particles

Particle samples were prepared by dusting particles using a spatula on a clean glass slide or slide coverslip from a distance of approximately five centimeters. The glass slide/coverslip was then shaken and tapped on the bench top to further aid in breaking up agglomerates and removing unattached particles. A minimal amount of sample should be used to limit the chances of agglomerates pending particle electrostatics.

Another method of preparing the particle slides, involved smearing them across the slide/coverslip using a clean spatula. The smearing action dragged agglomerates across the slide/coverslip producing individually attached particles. Both methods are sufficient in preparing glass slides or coverslips that contain single particles even though agglomerates will still be present. These methods are sufficient for loosely attaching single particles of up to 10 μm onto the glass slide even when inverted. Glass slides should be thoroughly wiped with lint-free kim-wipes to limit accidental attachment of dust or fine glass particles to AFM cantilevers.

2.3. Production of glass slide with epoxy

Equal amounts of the two component epoxy were expelled onto a clean glass slide and mixed thoroughly using a sterile pipette. A small amount of the mixed epoxy was smeared onto another clean glass slide to prepare the epoxy slide. It is important to keep the height of the epoxy minimal (a very thin smeared layer is sufficient). Ideally one should be able to focus on the epoxy and the glass slide simultaneously. A clean tool or slow stream of nitrogen can be used to further spread the epoxy on the 'epoxy slide' to achieve a thin epoxy layer of minimal height.

2.4. Fabrication of colloidal probe

The six step procedure shown in Fig. 2 was implemented as follows:

Using double-sided sticky tape, a tipless cantilever was first positioned on the edge of a custom designed 45° angle cantilever holder (Fig. 2 #1) and a prepared epoxy slide was attached to a custom designed microscope slide holder (Fig. 2 #2). During method optimization, other angled holders including 30°, 40°, 50°, and 60° were also tested. The height of the glass slide holder was adjusted by sliding it up or down the microscope lens casing to focus on the particles or epoxy (Fig. 2 #3). A re-usable adhesive such as Blu-Tack® was used on one edge to allow temporary attachment and easy exchange of particle and epoxy slides. The epoxy slide was attached (epoxy side facing down) so that half of the microscope field of view was glue and the other half blank slide (Fig. 2 #5). The cantilever mounted on the 45° holder (Fig. 2 #4) was then brought into contact with the edge of the epoxy layer and dragged in the XY plane out of the epoxy across the clean glass slide region to minimize the amount of and localize the epoxy onto the cantilever apex (Fig. 2 #5). Once glue was acquired on the cantilever, the epoxy slide was replaced with the particle slide and the slide holder readjusted to bring the particles into focus finding a single particle of interest (Fig. 2 #6). The