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Hybrid core/shell microparticles and their use for understanding biological processes

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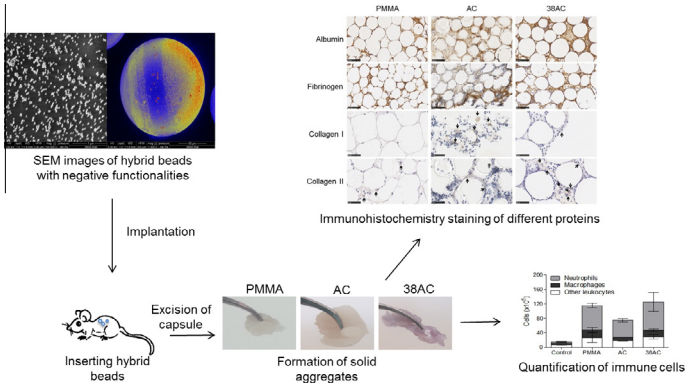
GRAPHICAL ABSTRACT



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ABSTRACT

Hybrid micro and nanoparticles have become a topic of intense research in recent years. This is due to the special properties of these materials that open new avenues in advanced applications. Herein, we report a novel method for the generation of hybrid particles utilising plasma polymerization. Poly (methyl methacrylate) (PMMA) beads were first coated with a thin allylamine based plasma polymer layer. Gold nanoparticles of engineered size and surface structure were then attached in a controlled manner to the plasma polymer coated beads. To generate uniform chemistry on the outermost surface and to preserve the nanotopography, we deposited a 5–10 nm thin layer of Acpp. We demonstrated that these particles can be utilized in *in vivo* models to interrogate important biological phenomena. Specifically, we used them in mice to study the inflammatory and foreign body responses to surface nanotopography. The data strongly indicates that surface nanotopography and chemistry can modulate collagen production

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Hybrid particles
Macrophages
Neutrophils

and the number of adhering immune cells. The method for generating hybrid particles reported here is solvent free and can open new opportunities in fields such as tissue engineering, drug delivery, biosensors, and regenerative medicine.

1. Introduction

Recently, hybrid micro and nanoparticles have become a hot topic of research because of the vast opportunities that they provide in many areas of research and technology [1–4]. The unique properties of these materials have led to the development of advanced applications which extend from medicine to photonics and optoelectronics [5–9]. The capacity of hybrid particles to influence biological systems has also led to innovative developments in drug delivery, tissue engineering, medical imaging and size exclusion chromatography [10–16]. Alternatively, porous silicon-based hybrid particles have been used in photonic devices [17] and Walnut-like CdS micro-particles/single-walled carbon nanotube hybrids in sensors and optoelectronics [18]. Core shell microparticles allow encapsulation of active molecules, extending their application to areas such as drug carriers, energy storage, transducers and dielectrics for electronics [19–22]. Hybrid silver/starch and silver/PLLA polymer nanocapsules and nanoparticles prepared by miniemulsion polymerization have been reported for their antibacterial properties and potential to serve as carriers of loaded drugs [23,24]. Double shell hybrid microparticles have been shown to enhance photocatalytic activity [25], further broadening their use for different applications.

The advanced properties and wide area of applications of hybrid particles have set the need for new fabrication techniques. Such techniques include layer by layer (LBL) deposition, precipitation co-polymerization, miniemulsion polymerization, electron beam lithography (EBL), self-assembly, and electrospinning [16,26–32]. These techniques yield interesting and useful materials, however most are complex, time consuming and have specific limitations. For example, layer by layer deposition on colloidal templates has been employed for synthesizing hollow particles and hybrid capsules. These are based on electrostatic assembly of negatively charged nanoparticles and positively charged molecules [30]. Despite their versatility, LBL is limited to particle surfaces with specific functionalities such as those having charged surfaces.

In this paper, we report a novel method for the generation of hybrid particles using plasma polymerization. A key benefit of the technique is that it can be applied to any type of substrate material as the technique is practically substrate independent and no surface pre-modification is required [33–36]. In contrast, self-assembled monolayers (SAM) require specific starting materials, LBL requires charged surfaces with multiple layers, and atomic layer deposition (ALD) can produce limited surface chemistry [33]. Another advantage of using plasma polymerization is that it is pin hole free, and conformal thin films can be deposited by using a single step coating procedure [37,38]. The process is also solvent free which is beneficial in terms of cost since it does not produce waste solvents. To generate hybrid particles, we attached to the plasma polymer coating gold nanoparticles of controlled size and appropriate surface functionalities as reported on planer surfaces [39]. PMMA beads were chosen because they are light and can be easily dispersed and injected in animals for the purpose of the biological studies reported below. We have developed comparative coatings on other types of particles such as silica. However, silica particles are heavier and difficult to inject since they block the needle of the syringe. We have chosen gold nanoparticles as they can be easily synthesized nearly monodispersed and are chemically inert in the size range used in this study [40].

To demonstrate the application of these hybrid microparticles, we utilized them to examine the immune and foreign body responses to surface nanotopography *in vivo*. It is known that surface nanotopography can play an important role in regulating cellular responses [8,41–45]; however model systems where these physiological responses can be studied *in vivo* are lacking. In the following study, we demonstrate the preparation of hybrid polymer/metal nanoparticles with well-defined nanotopography and chemistry, and their application in an *in vivo* model of acute biomaterial-induced inflammation.

2. Experimental section

2.1. Materials

Allylamine (AA) (98%, Aldrich), acrylic acid (Ac) (99%, Aldrich), hydrogen tetrachloroaurate (99.9985%, ProSciTech), trisodium citrate (99%, BHD Chemicals, Australia Pty. Ltd.), and 2-mercaptosuccinic acid (97%, Aldrich), were used as received. Poly (methyl methacrylate) (PMMA) non-functionalized beads (Bangs Labs, BB05N/5438) were obtained as a dry powder of microspheres of 125–180 μm diameter with a mean diameter of 153 μm.

2.2. Plasma polymerization

Plasma Polymerization was carried out in a custom built reactor with a 13.56 MHz plasma generator [46]. To allow for coating particles or beads a shaking device was added inside the plasma reactor as previously reported [47]. Deposition of allylamine and acrylic acid was carried out at a precursor pressure of 0.2 mbar and a deposition time of 5 min. Power used for deposition of both monomers was 40 W and 10 W, respectively. To achieve an overcoating of 5 nm, the time of deposition for was kept to 45 s. Before deposition, all beads were cleaned by applying air plasma for 2 min at 50 W.

2.3. Synthesis of gold nanoparticles (AuNPs)

Gold NPs were synthesized by reducing hydrogen tetrachloroaurate ($HAuCl_4$) using trisodium citrate. A 50 mL solution of 0.01% $HAuCl_4$ was brought to boiling temperature with vigorous stirring. Under vigorous stirring 1% water solution of trisodium citrate (TSC) was added. To achieve particles sizes of 16, 38 and 68 nm diameter, the amount of added TSC was 1 mL, 0.5 mL and 0.3 mL, respectively. Upon addition of trisodium citrate, the colour of the solution changes from light yellow to wine red within minutes. The solution was kept at boiling temperature for another 20 min, after which, it was left to cool to room temperature [48]. Once the nanoparticles were cooled, surface modification of these nanoparticles was performed using 2-mercaptosuccinic acid (MSA) as previously described [40].

2.4. Immobilization of gold nanoparticles

Plasma polymerized allylamine (AApp) coated PMMA beads were immersed for 2 h in 16 nm, 6 h in 38 nm and overnight in