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Nanopatterned protein-polysaccharide thin films by humidity regulated phase separation

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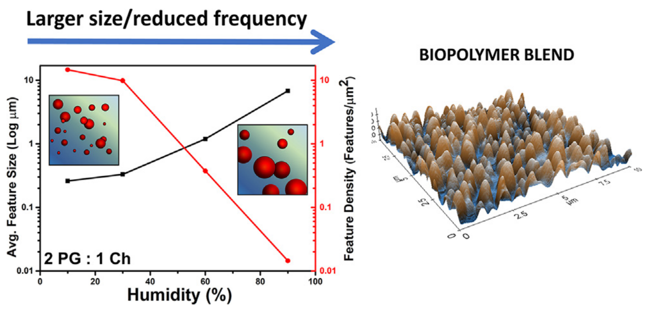
GRAPHICAL ABSTRACT



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ABSTRACT

Greater sustainability in mass manufacturing is essential to alleviating anthropogenic climate change. High surface-area, micro- and nano-patterned films have become a fundamental tool in materials science, however these technologies are subject to a dwindling petrochemical supply, increasing costs and disposability concerns. This paper describes the production of patterned biopolymer films utilizing controlled phase separation of biopolymeric thin films into nanopatterns using easily transferable variables and methods. Similar morphologies to those commonly observed with synthetic block-copolymers (BCPs) were achieved across a large range of feature sizes, from 160 nm to >5 µm: Bicontinuous, porous, droplet-matrix, particulated and dimpled. Protein and polysaccharide type, protein to polysaccharide ratio, casting method and ambient humidity were primary conditions found to influence the pore morphology of the films. High protein concentrations (4:1 and 2:1 blends) generally resulted in porous structures whereas high polysaccharide concentrations (1:2 and 1:4 blends) resulted in spherical structures. High humidity conditions (60% + relative humidity) resulted in the growth of large protuberances up to 10 µm in diameter while lower humidity (10–30%) resulted in discrete features smaller than 200 nm.

1. Introduction

Sustainable materials is a term that encompasses a sustainable, materials science based approach to technological development

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and product lifecycles. It focusses on bottom-up production methods; biodegradable, renewably sourced materials; and environmentally benign usage. The development of technologies from sustainable sources is essential to minimising negative anthropogenic effects on environment and climate. Resource finitude; waste management; and production and usage emissions need to be addressed. Our most crucial technologies must be prioritised for the initial developments of these new, sustainable materials.

Electronic and smart devices, and advanced medical materials; these are among our most vital modern technologies. High surface-area, micro- and nano-patterned films are fundamental to the manufacture and function of the aforementioned technologies, as well as finding widespread use in a variety of other applications. Apart from semiconductor [1] and medical tech [2], they are critical to superhydrophobic [3], anti-reflective [4,5] and self-cleaning materials [5]; anti-fouling coatings; and food texture technologies [6] (which will be of increasing importance as more and more synthetic food equivalents are required to replace environmentally unsustainable foodstuffs) [7]. The patterns are produced through phase separation of highly refined, synthetic, block-copolymer thin-films [8]. These polymers are a perfect example of a long term, unsustainable material. Sourced from petrochemicals, they will become prohibitively expensive with time, they are non-renewable, non-biodegradable, and the refinement processes of their production are environmentally damaging. Our research shows the development of a sustainable materials alternative; bottom-up production of patterned films from renewable, biodegradable biopolymers.

Biopolymers (proteins and polysaccharides primarily) are an ideal sustainable material, and an obvious alternative to conventional, petrochemical polymers in all but the most specialised applications [9]. They are abundant, readily accessible, renewable, compostable and; produced and extracted with minimal to no environmental impact [10]. Biopolymers also have suite of attractive features for manufacturers; high structural specificity; well-defined and varied functionalities; structure dependant solubility [11]; predictable viscosity [12]; bactericidal properties [13]; and biocompatibility [14]. Molecular weight distributions of polysaccharides can vary [15] but are readily refined [16] and in the case of proteins, monodisperse molecular weight distribution is an innate property. For all the above reasons, naturally occurring biopolymers have interested scientists and engineers for decades. They are used in food texturing [17], personal care products [18], cell binding [19], textiles [20] and membranes [21–23]. Many of these applications involve patterning of biopolymer surfaces; though the structures obtained have so far been much larger than those required for use in applications such as substrate patterning [24–29]. There are only a few notable examples of biopolymer blends being used to create surfaces with structures on a scale akin to those in this work [30–32]. However, these typically involve secondary etch steps, with harsh solvents and functionalised biopolymers to achieve desired morphologies, making them environmentally damaging.

Microphase separation phenomena in biopolymer-biopolymer-solvent systems falls into two categories; associative and segregative [33,34]. Categorisation is dependent on the affinity between the biopolymers and the solvent. Associative phase separation occurs when the biopolymers carry opposite charges, and segregative when they carry the same charge. Complexities arise in the form of; kinetic competition between gelation processes and phase separation process [35]; the influence of shear forces on formation mechanisms [36,37]; the influence of humidity on solution behaviours [38]; and the vagaries of biopolymer structure in solution, to name only a few. This paper reports phase separations of a specific type of biopolymer-biopolymer-solvent system; protein-polysaccharide-solvent solutions (hereafter referred to as the Pr-Ps-S solutions). These solutions are used to produce surface patterned, composite thin-films of polysaccharide and protein. Associative and segregative phase separations of such solutions have been studied extensively over the past four decades, primarily in food science. However, latter work has focussed on limited applications in packaging and biomedical devices [14,17,39,40].

Most scientific literature details phase separation of a variety of proteins and polysaccharides, generally in one type of solvent; water [33,41–44]. The exception is when the polysaccharide of choice is chitosan, when the solvent of choice is typically dilute acetic acid [45–47]. The research reported here builds upon state-of-the-art in the field of biopolymer phase separation in four ways: firstly, unique Pr-Ps-S solutions are studied; proteins are bovine serum albumin (BSA) and pigskin gelatin (PG), polysaccharide is chitosan (Ch), and solvent is formic acid (FA). Secondly, the production of thin-films from these Pr-Ps-S systems is examined with a view to their use in materials applications beyond the food, packaging and biomed industries. Specifically, utilising micro- and nanopatterns in templating, and smart textiles applications. Thirdly, the control of formation conditions under which these phase separations occur is different. Control of conditions such as humidity and ambient temperature is usually more associated with the formation processes of synthetic polymer solutions for medical device and advanced membrane applications [48–51]. Control of biopolymer phase separation of the Pr-Ps-S solutions confers a degree of control over the morphology final thin-films, affecting utility. Fourthly, the analytical data concerning the growth of surface features of the biopolymer thin-films is compared to that of Ostwald ripened structures. The findings described in this paper shows that controlled phase separation of biopolymer blends is an effective method of producing micro- and nano-patterned surfaces.

2. Experimental

2.1. Biopolymers, casting solutions and substrate

Low molecular weight chitosan (50–190 kDa) > 75% deacetylation, high bloom gelatin from porcine skin (~300 bloom, Type A premium grade) and Bovine Serum Albumin (lyophilised powder, ≥96%, molecular weight ~66 kDa) were purchased from Sigma Aldrich. Substrates used in all cases were Fisherbrand™ Microscopic Slides with Ground Edges (plain). The solvent used was Formic acid, 98+ %, pure, ACROS Organics™ and was diluted to 90% w/v before use using distilled water. Casting solutions were prepared using 90% formic acid as solvent to ensure that the biopolymers above were below their isoelectric point in solution and so, positively charged. This was to ensure that any phase separation processes occurring in the Pr-Ps-S solutions were segregative.

2.2. Solution preparation

Prior to dissolution, proteins and polysaccharides were dried overnight at room temperature under vacuum. Polymer stock solutions were made by solubilising chitosan (Ch), bovine serum albumin (BSA) and pigskin Gelatin (PG) in 90% formic acid (FA) acid at 5 w/v% 10 w/v% and 10 w/v%. These solutions were stirred in a closed vessel for 3 hr in a closed container at room temperature. The solutions were then centrifuged at 13,000 rpm in a Beckman Coulter Avanti J-26XPI centrifuge at 18 °C for 15 min and decanted. Following this, stock solutions were stored at −20 °C for further use or used immediately. Stock solutions were diluted with fresh formic acid and/or mixed with each other to produce coating solutions.