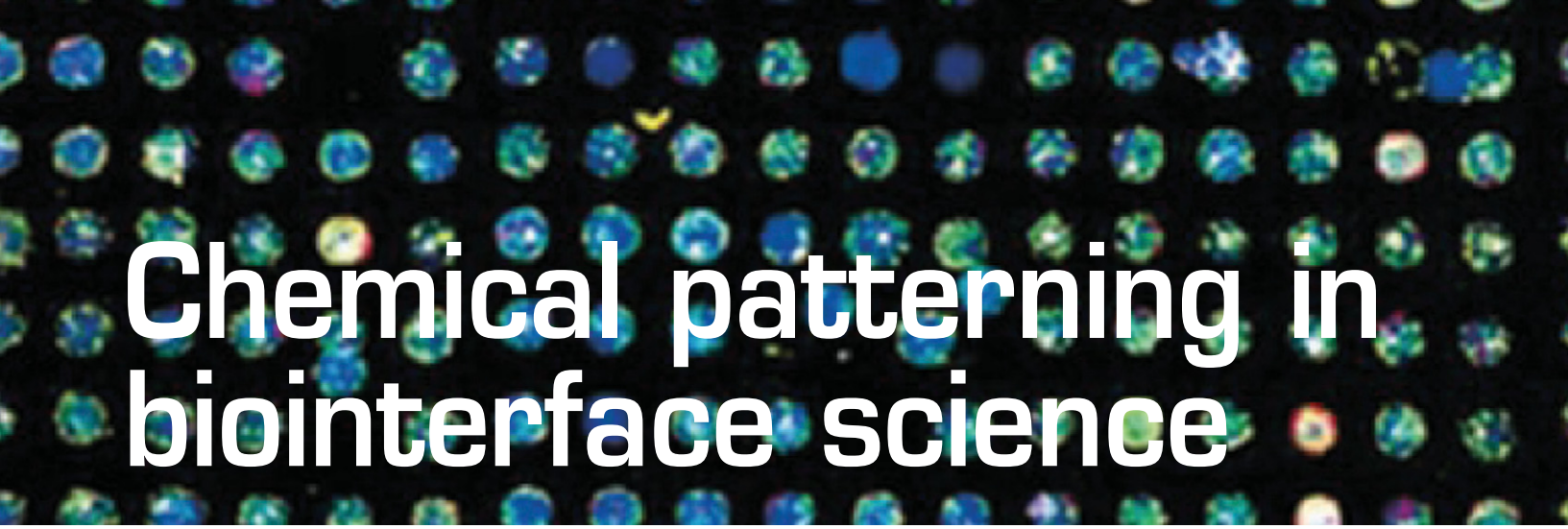

Chemical patterning in biointerface science

Patterning of surfaces with different chemistries provides novel insights into how proteins, cells and tissues interact with materials. New materials, and the properties that their surfaces impart, are highly desirable for the next generation of implants, regenerative medicine and tissue engineering devices, and biosensors and drug delivery devices for disease diagnosis and treatment. Patterning is thus seen as a key technology driver for these materials. We provide an overview of state-of-the-art fabrication tools for creating chemical patterns over length scales ranging from millimeters to micrometers to nanometers. The importance of highly sensitive surface analytical tools in the development of new chemically patterned surfaces is highlighted.

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As society strives towards an improved quality of life, we care for an ever increasing elderly population and attempt to combat the increase in life-style associated health conditions, such as diseases of the cardiovascular system. This drives researchers to develop innovative biomaterials with new and improved surfaces. In most cases the materials used to treat and diagnose disease are in contact with biological fluids that contain proteins, which spontaneously and irreversibly adsorb to surfaces. It is this surface that cells contacting the material 'see', often triggering material failure[1-6]. Cells respond to the biochemical signals contained within the peptide motifs of the adsorbed proteins through extra cellular matrix receptors such as integrins[7]. The consequences of uncontrolled protein adsorption on man made surfaces can be very severe indeed, resulting in not only device failure but also additional health risks to patients[8]. Examples include:

1) attachment and colonisation of pathogenic bacteria to venous catheters via adsorbed protein layers that can lead to high patient mortality[9];
2) thrombosis on the surfaces of cardiovascular devices such as artificial hearts, catheters and prosthetic valves from platelet adhesion and activation[10];
3) fouling of hemodialysis membranes[11]; and
4) inflammatory responses that lead to restenosis following the insertion of stents[12].

On the other hand, the fields of tissue engineering[13], biosensors and diagnostic arrays[14,15] and drug delivery systems[16], which

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aim to regenerate, diagnose and treat diseased tissues, rely on proteins and biomolecules being presented either to attach cells in the aqueous environment in the correct conformation and spatial configuration in 1, 2 and 3D to optimize bioactivity and minimize adverse reactions.

The realization that interfacial phenomena were important to biomaterials came after World War II when the concept of 'biocompatibility' emerged[17]. This lead to acceleration in the field of surface and interface research targeted at biology and medicine. The progress made has encouraged scientists and engineers to pursue these goals further with the development of chemically patterned surfaces with nanometer-scale precision, with a vision of ultimately fabricating and controlling the biological system as desired through the interface with the man made materials. The birth of a new field, termed 'nanobiotechnology' arose, which utilizes biological systems to fabricate functional nanostructured and mesoscopic architectures comprised of organic and inorganic materials[18]. One of the fundamental biological phenomena that has driven and continues to underpin this field is the fact that cells respond to topographical and chemical cues from their environment by interacting with extracellular matrix (ECM) and other cells *in vivo*, and mechanical and chemical properties of material-surface interface *in vitro*. The subsequent cell signaling events ultimately influences cell function, shape, migration, adhesion, survival, proliferation and differentiation[19-24].

To understand the complex relationship between the surface chemistry and biological systems, there has been a focus on combining topographical and chemical modification to create multi-functional surfaces. Highly defined topographical and chemical features from the mm to nm range are intended to span the length scales of tissues to cells to proteins and other biomolecules – these synthetic 'models' of biology will allow us to rationally study and comprehend the complex interfacial behavior possessed by the biological system in 2- and 3D. The ability to mimic biological surfaces permits us to unravel the essential controlling factors in biology by studying the interaction between the mimicked surface and biological components.

Early developments of surface fabrication were inspired by the 'top down' approaches, traditionally used in microelectronics such as photolithography and electron beam (e-beam) lithography[25-27] for devices such as microprocessors[28], MEMS[29] and NEMS[30]. Typically it is possible to obtain a surface topographical feature of down to ~10 nm with e-beam lithography. Techniques such as Transmission Electron Beam Ablation Lithography (TEBAL)[31] have been recently developed in an attempt to conquer the challenge of reaching the sub-10 nm resolution limit. TEBAL is carried out by controllably ablating evaporated metal films, pre-patterned with e-beam lithography on silicon nitride membrane substrates, to produce a variety of intricate nano-features such as gaps, rings, channels and wires. Many technological methods for surface fabrication have been inspired by the techniques from the printing industry; for instance, ink-jet technology has a number of potential life science applications such as genomics, combinatorial chemistry, drug discovery and tissue engineering[32-34]. Current jet printer technology has reached the limit of printing high resolution features on a surface down to ~ 1 μm in a parallel manner[35].

Surfaces can be fabricated by 'bottom up' approaches, for example, molecular self-assembly such as alkane thiols on gold is a commonly used method to form well ordered surfaces. A range of surface functionalities can be presented on the surface by introducing different chemical terminal groups either as a single component or multiple components[36-40]. Certain types of polymers such as block copolymers, and particulate systems, can self assemble into a variety of nanoscopic structures with topographical scales of 5 to 50 nm[41-43]. Examples of how dimensions influence cellular behavior come from the Spatz group. They have identified critical surface spacing ranges for the cell-adhesion peptide sequence RGD, which influence the structure of integrin receptors on cell surfaces. A spacing on 58 nm vs 108 nm showed large variations in integrin mediated cell behaviour. On 58 nm RGD patterns cell spreading was delayed and motility was erratic, whereas on 108 nm patterns cell adhesion sites exhibited rapid turnover indicating a critical RGD density was necessary for develop stable integrins, and efficient cell spreading and focal adhesion formation[44]. Furthermore, disordered patterns of > 70 nm cyclic RGD were found to be necessary to 'turn-on' cell attachment compared to ordered 70 nm patterns that 'turned off' attachment[45]. These nano dimensions have been related to the size of the integrin structural elements indicating that cell adhesion is sensitive to dimensions much smaller than the size of an individual cell. Another emerging 'bottom up' fabrication method is the implementation of micro and nanometer sized colloidal particles known as 'colloidal lithography'. Recent advances in colloidal synthetic methods have enabled highly monodisperse particles to be produced with good phase stability. Two dimensional structures with lateral feature sizes in the range of micrometers to nanometers can be readily assembled simply by selecting different sized particles. By using colloidal patterns as masks, diverse topographical features and geometrical control can be obtained; either directly by reactive ion etching (RIE) on single and multi-layered colloidal surface[46,47], or by depositing various organic and inorganic materials on a substrate surface through the interstitial spaces formed between the particles via evaporation or sputtering followed by the subsequent removal of particles[48,49]. A novel method 'shadow nanosphere lithography' is capable of creating a range of topographical morphologies such as cups, rods and wires in the nanometer size range on the surface by varying the position and angle of the substrate with respect to the evaporation source[50,51]. Various 'bottom up' and 'top down' fabrication methods have been also employed in combination for surface patterning. Non-photolithographic techniques including various soft lithography methods[52-56], dip pen nanolithography (DPN)[57] and nanoimprint lithography[58] have been developed over the last two decades.