

chemotactic gradients of soluble and insoluble proteins) and/or physical (i.e., aligned fibres or stiffness gradients) environments during processes such as embryogenesis and wound healing (Perez-Castillejos 2010). Hydrogels are being designed to recreate such environments.

Strategies to create topographical anisotropy

Given the importance of surfaces in most biomaterials, a relatively simple way to provide physical anisotropy to hydrogels is through surface topographies (Verhulsel et al. 2014). Topographical patterns can be easily fabricated with a high level of precision thanks to techniques derived from microlithography (Whitesides 2005) including nanoimprint lithography, electron beam lithography, focused ion beam lithography (Smith et al. 2011). Of all, however, the most important and influential technique that has been exploited to create surface patterns is soft lithography due to its simplicity, accessibility, and versatility (Xia and Whitesides 1998). This method has been extensively exploited to create surface topographies on a large variety of hydrogel materials and subsequently provide an additional level of functionalization.

Chitosan substrates (Fukuda et al. 2006b), hyaluronic acid materials (Fukuda et al. 2006a), or elastin-like polymer membranes (Tejeda-Montes et al. 2012; Tejeda-Montes et al. 2014) have been topographically patterned using soft lithographic techniques to guide cell behaviour in different ways through physical hydrogel surface anisotropy. Other methods have been developed for example taking advantage of the surface folds that form during shrinkage of PHEMA-based hydrogels, which were shown to promote osteoblastic differentiation (Guvendiren and Burdick 2010) or photopatterning dynamic PEG-based hydrogels capable of modifying surface topographies in real time and studying how cells respond to these changes (Kirschner and Anseth 2013). The combination of lithographic techniques, such as e-beam lithography and soft lithography, have been used to create surface topographies down to nanometer scales (Mata et al. 2009). However, hydrogels made from self-assembling molecules offer the possibility to provide surface topographies at different scales by taking advantage of the bottom-up assembly of nanostructures. In this way, a higher level of bioactivity and functionalization may be achieved. For example, combining soft lithography with self-assembling peptides enables the fabrication of various surface microtopographies, which are made from well-defined and organized nanofibres. By modulating the assembly process, it is possible to create surface microtopographies made from aligned nanofibres. This process may be exploited to fabricate hydrogels with surface topographies while co-assembling different molecules such as self-assembling peptides and hyaluronic acid (Mendes et al. 2013b) (Fig. 3a).

Strategies to create structural anisotropy

In spite of the capabilities to surface pattern hydrogel materials, these materials are particularly attractive to provide synthetic ECM-like environments for cell encapsulation. Therefore, there is great potential in creating physical and chemical anisotropy within the 3D hydrogel material. Different hydrogels can exhibit a spectrum of porosity and nano/micro-architecture. Those made from entangled fibres