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Screening of rat mesenchymal stem cell behaviour on polydimethylsiloxane stiffness gradients

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ABSTRACT

Substrate stiffness is emerging as an effective tool for the regulation of cell behaviours such as locomotion, proliferation and differentiation. In order to explore the potential application of this biophysical tool, material platforms displaying lateral and continuously graded stiffness are advantageous since they allow the systematic exploration of adherent cell response to substrate stiffness and the tuning of the material to elicit the desired cell behaviour. Here, we demonstrate a simple approach towards the fabrication of polydimethylsiloxane (PDMS) stiffness gradients (with an indentation modulus of 190 kPa–3.1 MPa across a 12 mm distance) by means of a temperature gradient during curing. We then apply these stiffness gradients to the screening of osteogenic differentiation in rat mesenchymal stem cells (rMSCs). Our proof-of-principle results show that mineralization of rMSCs is strongly dependent on the PDMS substrate stiffness, but is also influenced by the display of extracellular matrix proteins preadsorbed on the gradients. This screening capability holds tremendous potential for the design of improved implant materials and tissue engineering scaffolds.

1. Introduction

A key challenge in regenerative medicine, cell therapy and tissue engineering is the control of cell behaviour – in particular, the control of cell phenotype through the use of chemical, biological and physical tools [1]. One such tool is the stiffness of the underlying substratum, which has recently been shown to effectively regulate cell behaviours such as locomotion, proliferation and stem cell differentiation [2–4]. In vivo, tissues possess different stiffness values, ranging from ~1 kPa in soft tissues such as the brain [5] to ~10 GPa in stiff tissues such as bone [6,7]. Substrate stiffness has been studied as an important determinant of the fate of cells in vitro [8–11]. For example, fibroblast movement and spreading are more extensive on a relatively stiff substrate (30 kPa) than on a softer one (14 kPa) [4,10]. In addition, Engler et al. [12] showed that stiffer polyacrylamide (PAAm) substrata (25–34 kPa) induced greater expression levels of osteogenic marker expressions in human mesenchymal stem cells (hMSCs), whilst softer substrates (0.1–17 kPa) elicited increased myogenic or neurogenic expression. Rowlands et al. [13] achieved a similar outcome by directing hMSCs toward osteogenic and myogenic lineage on PAAm gels with different substrate stiffness (0.7–80 kPa) coated with adhesive proteins. PAAm hydrogel has also been used widely as a tunable mechanical property substrate [2,12,13].

Whilst these existing studies exemplify the potential of substrate stiffness as a powerful regulator of cell behaviour and cell fate, it is important to note that the majority of them investigated cell responses on substrates with uniform mechanical properties. Certain cellular phenomena, such as durotaxis, cannot be detected on such uniform substrates but can only be detected on substrates displaying gradients of mechanical compliance [4,14,15]. In order to explore the role of substrate mechanical properties on cellular phenomena, suitable biocompatible compliance gradient substrates are highly desirable. Radial compliance gradients made from PAAm gels have been fabricated for studies of cell–substrate interactions. Although these gradient gels were designed originally for the purpose of electrophoresis [16], they have been employed more recently in studies of cell locomotion [4,10,15]. For example, the study by Wong et al. [10] investigated migration and spreading of vascular smooth muscle cells on PAAm compliance gradients. The potential limitation of PAAm is that leaching of residual acrylamide monomer may cause cytotoxicity [17–20], and elastic moduli of PAAm gels are typically in the range of 1–100 kPa [2,10,12], which is lower than the native stiffness of most tissues [7]. Hence, a new protocol to fabricate compliance gradient substrates made from a more biocompatible material and supporting long-term study of MSCs differentiation is the key goal of the present study.

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