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A novel and simple microcontact printing technique for tacky, soft substrates and/or complex surfaces in soft tissue engineering

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ABSTRACT

Microcontact printing (μCP) has attracted much interest due to its simplicity and wide range of applications. However, when conventional μCP is applied to soft and/or tacky substrates, substrate sagging and difficulty in stamp removal cause non-conformance in the patterns. Moreover, it is almost impossible to apply conventional μCP on complex or wavy surfaces. In this study, we developed a novel yet simple trans-print method to create efficient micropatterning on soft and/or tacky substrates such as polydimethylsiloxane and polyacrylamide gel, and also on curved surfaces, by introducing polyvinyl alcohol film as a trans-print media. This technique is simple as it only involves one trans-print step and is also cost-effective. Most importantly, this technique is also versatile and we have proven this by printing various designs on more complex non-flat surfaces using various proteins as inks. The quality of the trans-printed pattern was excellent with high reproducibility and resolution as verified by immunostaining. Human mesenchymal stem cells cultured on these patterns displayed good conformance on the soft and tacky substrates printed using this technique. These results suggest that this novel trans-print technique can be extended to a potentially generic methodology for μCP of other proteins and biomolecules, other shapes and sizes, and cells, and will also be useful in three-dimensional micropatterning for soft tissue engineering.

1. Introduction

Many studies have reported on the close interplay between cell shape and stem cell differentiation [1,2]. For example, it has been shown that cell shape affects human mesenchymal stem cell (hMSC) differentiation, and that there is an interplay between cell shape and focal adhesion assembly [3]. On this front, surface patterning is usually required and microcontact printing (μCP), which is a top-down technique first described by Whitesides, remains one of the most popular methods of surface patterning [4]. While conventional μCP has proved satisfactory in the past, in recent emerging research involving soft tissue engineering, this technique is now inadequate [5]. It has been established that in soft tissue culture, the substrate materials for stem cell culture have to be mechanically compliant with the soft tissues [6,7]. These materials, such as polydimethylsiloxane (PDMS) and polyacrylamide (PA) gel, which have a Young's modulus in the range of 40 kPa or less, can be quite tacky, resulting in stamps adhering to the tacky substrates and distorting the patterns as illustrated in Fig. 1A–D. These drawbacks become more significant the softer and tackier the substrate. Fig. 1E illustrates the acceptable performance of conventional μCP on a 308 kPa hard PDMS substrate vs. the performance on soft and tacky substrates shown in Fig. 1F and G. Moreover, as conventional PDMS stamps are relatively stiff for ease of handling, it is also not easy to print on complex surfaces such as cylinders or spherical scaffolds. In theory, PDMS stamps could be sliced very thin to make them flexible, but in reality it is almost impossible to handle such thin stamps. Rape et al. reported a trans-printing method that involved introducing a coverglass to trans-print gelatin on 6 kPa soft PA gel [8]. This method is impressive and very similar to our work except that it requires an additional trans-print coverslip removal step. It is anticipated that this additional step may restrict the method from being applied to tacky soft PDMS substrates as the force of the removal may cause deformation of the soft substrates as indicated in Fig. 1. There have also been other attempts to resolve the issues mentioned above, but to date, these methods either could not solve all the issues or are costly, complicated and not versatile [9,10].

Given the escalating importance of matrix compliance and surface patterning for soft tissue engineering, we aim to establish a generic and versatile method which could achieve efficient μCP with a high resolution on soft and/or tacky substrates, and subsequently extend its use to geometrically complex substrates such as curved or wavy surfaces at a low cost and high resolution. In this technique, we use a sacrificial polyvinyl alcohol (PVA) film as a trans-printing media and demonstrate its efficiency with various proteins and pattern dimensions [11].

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