Regenerative Medicine & Cellular Therapies

Biomaterials Discovery

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CHEMICALLY FUNCTIONALISED AND TOPOGRAPHICALLY TEXTURED MICROPARTICLES TO SCREEN STEM CELL-PARTICLE INTERACTIONS

M. Alvarez-Paino[1], M. Amer[1], K. M. Shakesheff[1], D. Needham[2], M. R. Alexander[1], C. Alexander[1] and F. R. A. J. Rose[1]*

[1]School of Pharmacy, University of Nottingham, Nottingham, UK [2]Department of Mechanical Engineering and Material Science, Duke University, Durham, North Carolina, USA

INTRODUCTION

Stem cell technologies require suitable biomaterials capable of supporting cell proliferation and modulating cell response. Biomaterials design therefore becomes crucial for a successful regenerative medicine therapy. It is well-known that material properties, such as chemistry[1], topography[2] and elasticity[3], are capable of inducing particular cellular behaviours and individually control stem cell fate. The aim of this work is to study this concept on large libraries of 3D materials which combine all these properties by fabricating a series of chemically modified, textured microparticulate architectures.

METHODS

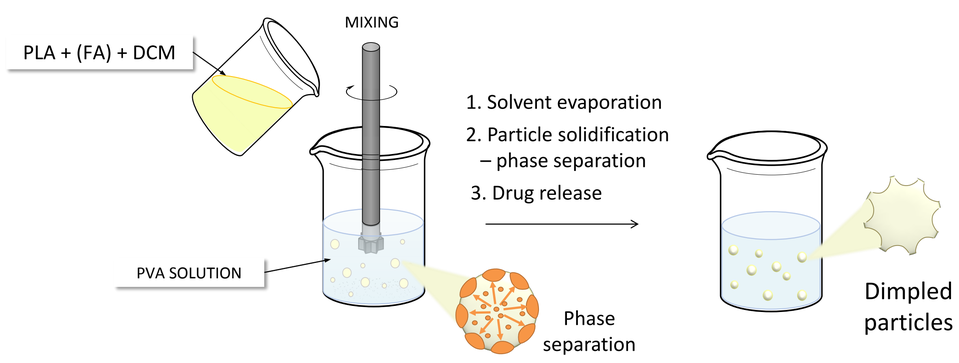
Poly(lactic acid) (PLA) (74 kDa) microparticles (MP) were prepared by oil-in-water emulsification of PLA solution in dichloromethane (DCM) in a 1% w/v polyvinyl-alcohol (98% hydrolysed, 13-23 kDa) aqueous solution. Then, MP were treated with ethylenediamine solution (0.5M in isopropanol) and α-bromoisobutyryl bromide was subsequently reacted with the amino groups. Finally, 2-hydroxyethyl methacrylate (HEMA) was grafted from the surface of these MP.

Alternatively, the drug fusidic acid (FA) was added to ~~in~~ the organic phase at different polymer/FA ratios and emulsification was performed as described. Dimpled particles were obtained after FA release during 7 days in PBS.[4]

Immortalised human mesenchymal stem cells (iMSCs) were seeded onto the unmodified MP either statically or dynamically, and their attachment, viability, and aggregation were assessed using Live/Dead staining and scanning electron microscopy (SEM).

RESULTS

MICROPARTICLE FABRICATION



Scheme 1. Schematic representation of microparticle fabrication using a drug-induced phase separation oil-in water emulsion process.

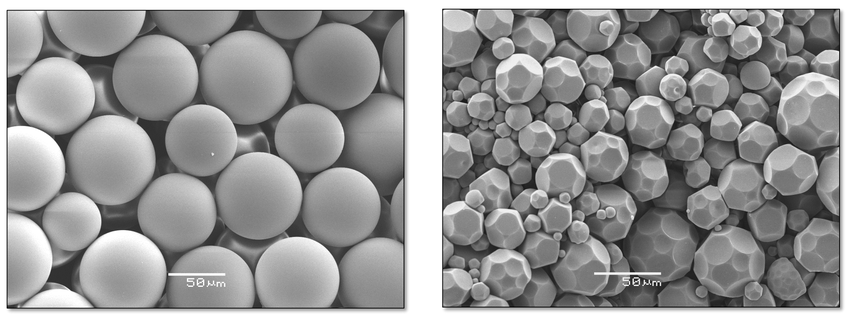


Figure 1. SEM images of smooth (left) and textured (right) PLA MP fabricated using the emulsion technique.

- The phase separation process generated particles with a characteristic 'golf ball'-like pattern on the particle surface. Also, the dimple size can be tuned by varying the amount of FA added to the initial polymer/drug mixture.

3D CELL CULTURE

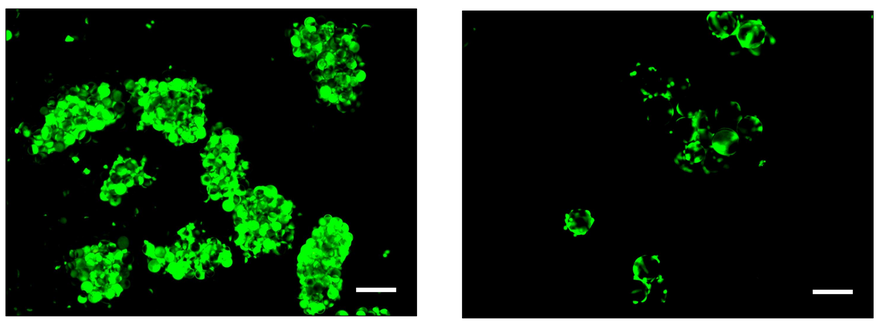
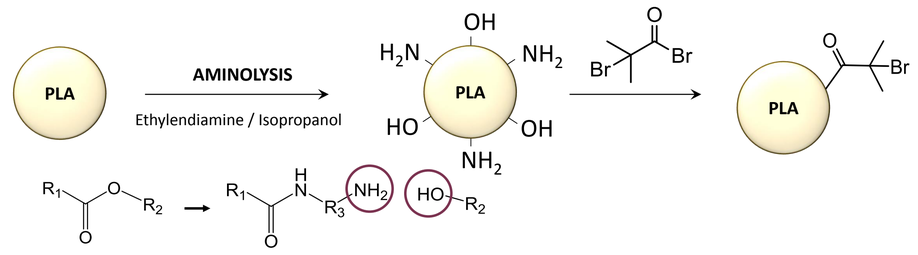


Figure 2. Representative fluorescence images showing iMSCs, stained using Live/Dead assay, seeded on microparticles of various sizes (Left: MPs with average size of 70 µm; Right: MPs with average size of 130 µm). Scale bar: 100 µm

- Seeding iMSCs (green) on the microparticles resulted in the formation of cell-particle aggregates (Figure 2) independent of seeding conditions employed (static *vs* dynamic). However, particle size had an influence on aggregate formation, with larger particles resulting in smaller aggregates.

MICROPARTICLE FUNCTIONALISATION

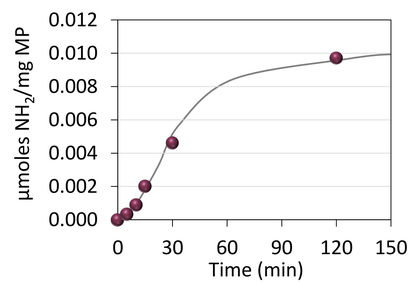


Scheme 2. Schematic representation of particle surface activation via aminolysis and initiator post-coupling.

- MP were aminolysed in order to enhance surface functionality and reactivity. In a second step, an ATRP initiator was coupled to the amino groups (Scheme 2). The extent of aminolysis and the success of the initiator immobilisation were assessed by the 2,4,6-trinitrobenzene sulfonic acid colorimetric assay (Figure 3A).

A

Time (min)	µmol NH_2/mg MP
0	0.0000
5	0.0003
10	0.0009
15	0.0020
30	0.0046
120	0.0097



B

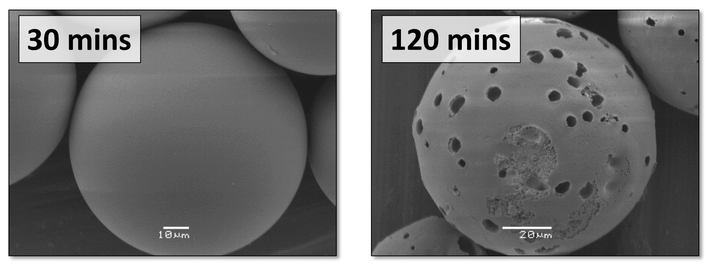
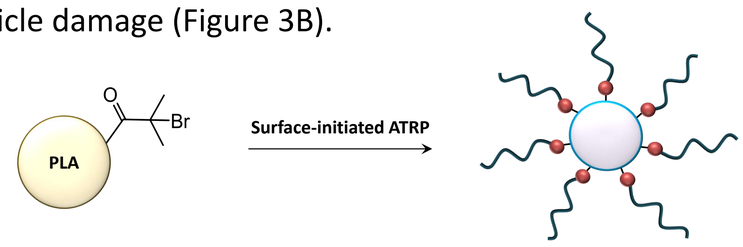


Figure 3. A) Particle functionalisation depending on the aminolysis time represented as the amount of NH_2 groups per milligram of MP. B) SEM images showing the effect of the aminolysis treatment length on particle integrity.

- Control of the particle exposure time to aminolytic regents is essential not only in order to achieve greater functionalisation, but also in order to avoid particle damage (Figure 3B).



Scheme 3. Schematic representation of polymer grafting from surface-activated MP.

- Monomers of interest can be subsequently polymerised from the activated surfaces. HEMA was polymerised from the surface of these MP demonstrating the success of this approach to obtain functionalised MP.

CONCLUSIONS

- In this work, chemically and topographically modified microparticles have been presented.
- The methodologies selected allow the incorporation of functionalities of a very dissimilar nature by following a common approach from a single core material. Also, bulk material properties and the topographical features could be retained whilst changing the outermost surface characteristics.
- In conclusion, these materials present a platform for new biomaterials discovery in 3D for potential regenerative medicine applications. In the future, topo-chemical combinations on cardiomyocyte maturation and mesenchymal stem cell differentiation in 3D will be evaluated.

REFERENCES

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