Regenerative Medicine & Cellular Therapies

Biomaterials Discovery

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

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INTRODUCTION

Understanding biomaterial-cell interactions is important in order to establish the basis for successful tissue engineering and consequently improve the capacity for tissue repair. Material properties including topography,[1] chemistry[2] and stiffness[3] have been demonstrated to individually have an impact on mammalian 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.

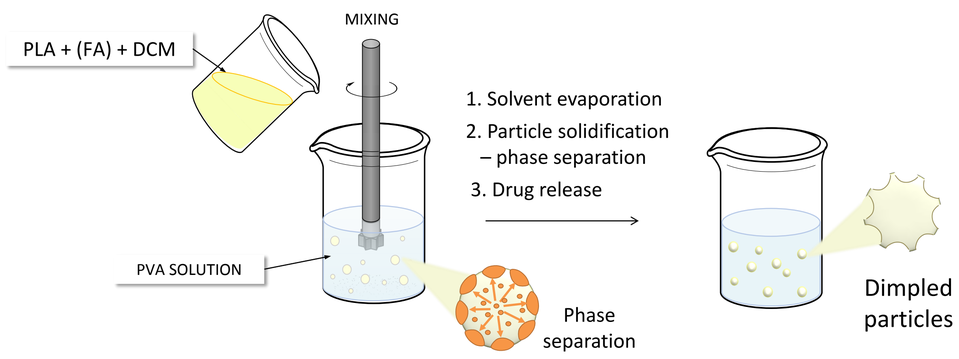
METHODS

Poly(lactic acid) (PLA) and poly(lactic acid-co-glycolic acid) 85:15 (PLGA) microparticles (MP) were fabricated by solvent evaporation oil-in-water single emulsion. Polymer dissolved in dichloromethane (DCM) was emulsified in the aqueous phase in the presence of polyvinylalcohol (PVA, 98% hydrolysed, 13-23 kDa) as a stabiliser (1% w/v). 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 above. Dimpled particles were obtained after FA release during 7 days in PBS.[4]

RESULTS

TEXTURED MICROPARTICLE FABRICATION



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

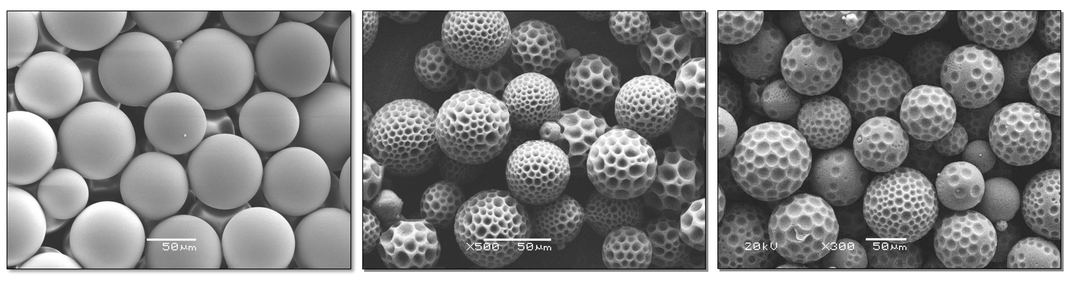


Figure 1. SEM images of smooth PLA (left) and textured PLGA (middle) and PLA (right) MP fabricated using the emulsion technique.

- The phase separation process generated particles with a characteristic 'golf ball'-like pattern on the particle surface (Figure 1). The average particle diameter varied by changing emulsification conditions.

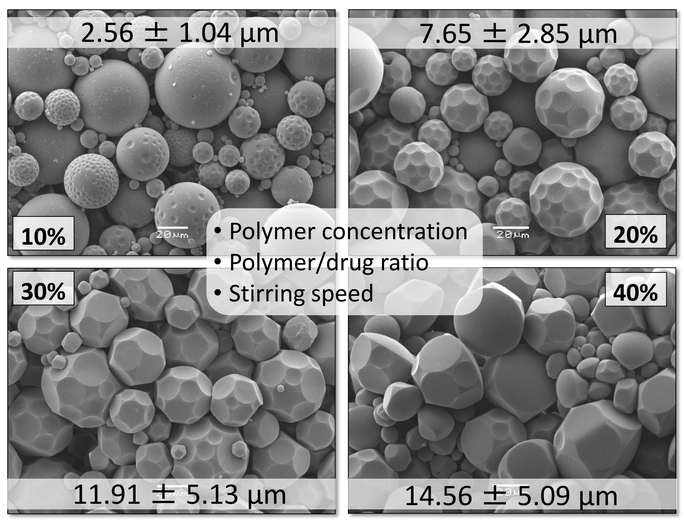
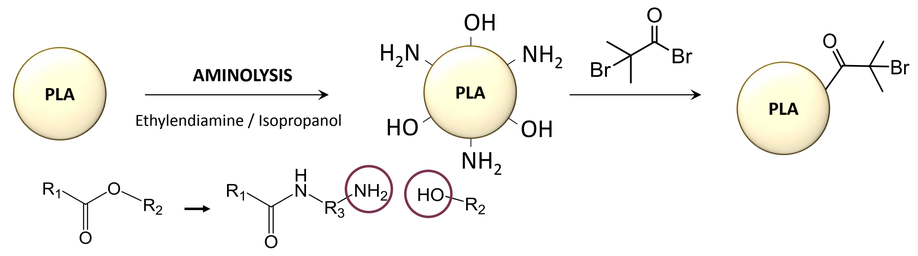


Figure 2. Effect of drug content on dimple formation and dimple size in PLA MP. Percentages indicate amount of FA in the initial polymer/drug ratio. Values indicate average dimple size ± S.D. Average particle sizes 45 µm.

- Polymer and drug concentration in the initial mixture significantly influenced the final particle and dimple size distribution. In addition, dimple size can be tuned by varying the initial polymer/drug ratio and increases with the drug content in the feed (Figure 2).

MICROPARTICLE FUNCTIONALISATION

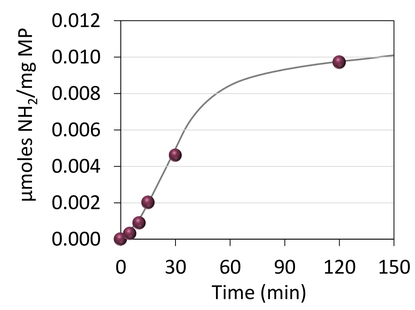


Scheme 2. Schematic representation of particle surface activation via aminolysis and initiator post-coupling.

- MP were aminolysed in order to enhance surface functionality and reactivity. In a second step, 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

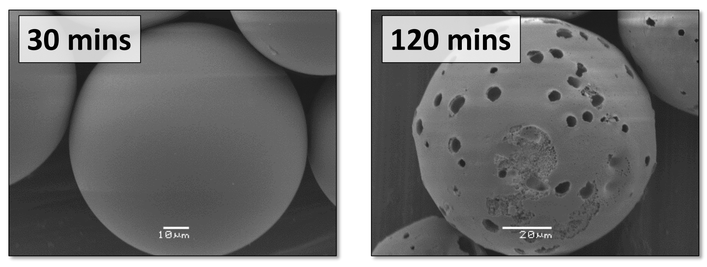
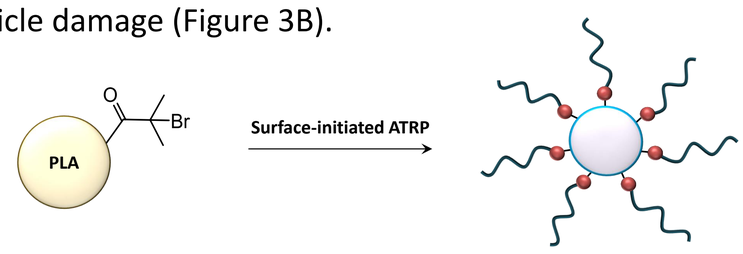


Figure 3. A) Particle functionalisation depending on the aminolysis time represented as the amount of NH_2 groups per milligram of MP. B) SEM images showing the effect of the aminolysis treatment length on particle integrity.

- 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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