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Biomaterials Discovery

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CHEMICALLY-MODIFIED MICROPARTICLES TO ENHANCE INITIAL STEM CELL ADHESION AND GROWTH

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INTRODUCTION

Material properties including topography[1], chemistry[2] and stiffness[3] are capable of modulating cell response. In tissue engineering, understanding biomaterial-cell interactions is crucial in order to improve the capacity for tissue repair. In this work, we aim at identifying new functionalities that could induce stem cell differentiation. To achieve this, the surface of biodegradable microparticles have been modified to study the effect of surface chemistry of 3D materials on stem cell response as a preliminary step for further studies with more complex chemical modifications.

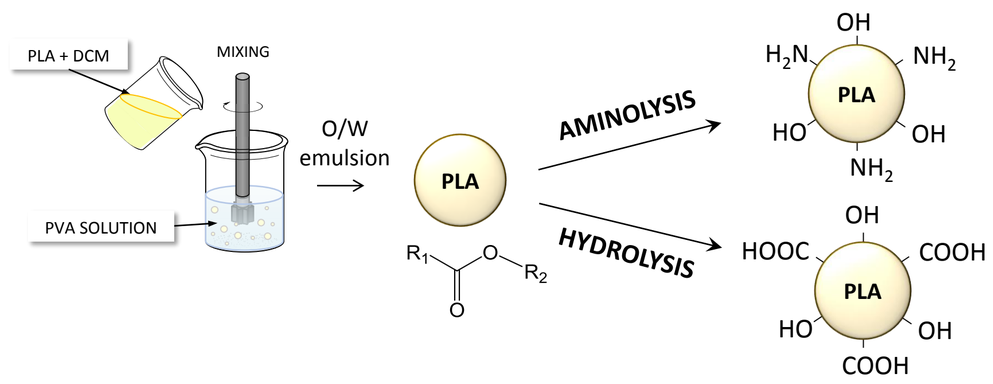
METHODS

Microparticles (MP) of poly(lactic acid) (PLA) (74 kDa) were fabricated by solvent evaporation oil-in-water single emulsion. Polymer dissolved in dichloromethane (DCM) (20% w/v) was emulsified in the aqueous phase in the presence of polyvinylalcohol (PVA, 98% hydrolysed, 13-23 kDa) as a stabiliser (1% w/v). Agitation speed was varied from 1500 to 2500 rpm to target different particle sizes. Then, MP were treated with ethylenediamine solution (0.5M in isopropanol) or sodium hydroxide solution (0.5M 70/30 isopropanol/water).

HUES7 cells (human embryonic stem cell, hESC) were seeded as single cells at 60 kcells/well on MP placed in ultra-low attachment well plates. E8 medium containing ROCKi and inter-alpha inhibitor was used. After 5 days of culture, cells were fixed and immunostained.

RESULTS

MICROPARTICLE FABRICATION

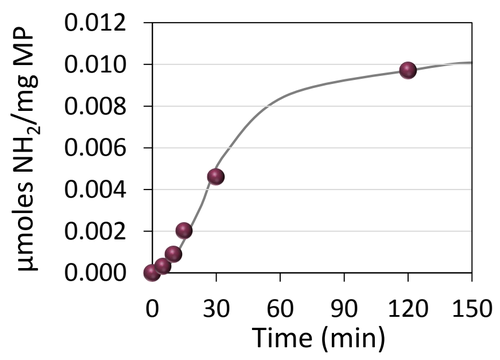


Scheme 1. Schematic representation of particle surface activation via aminolysis and hydrolysis.

MP were aminolysed and hydrolysed in order to enhance surface functionality and reactivity (Scheme 1). The extent of aminolysis was 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

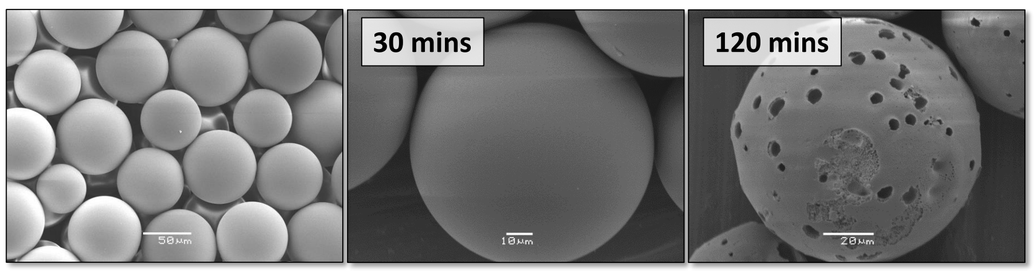
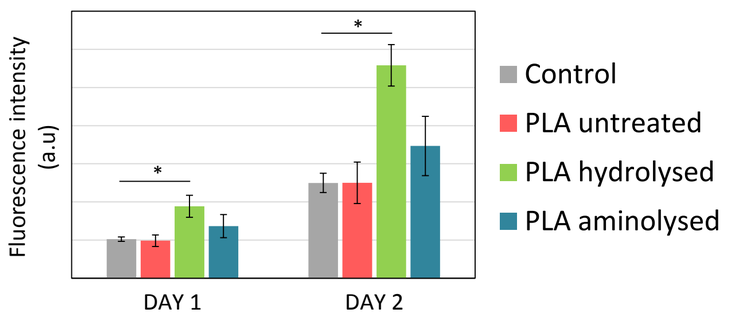


Figure 1. A) Particle functionalisation depending on the aminolysis time represented as the amount of NH_2 groups per milligram of MP. B) SEM images showing smooth particles prepared by the emulsion technique and the effect of the aminolysis treatment length on MP integrity.

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

3D CELL CULTURES

Figure 2. Cell metabolic activity determined by Alamar Blue assay. Control: cells cultured as embryonic bodies. Particles: 50-100 µm. Statistical analysis: t test ($p < 0.05$)



Cells seeded on hydrolysed particles showed the highest metabolic activity, followed by the cells grown on aminolysed particles. Cells seeded on untreated particles showed the lowest metabolic activity.

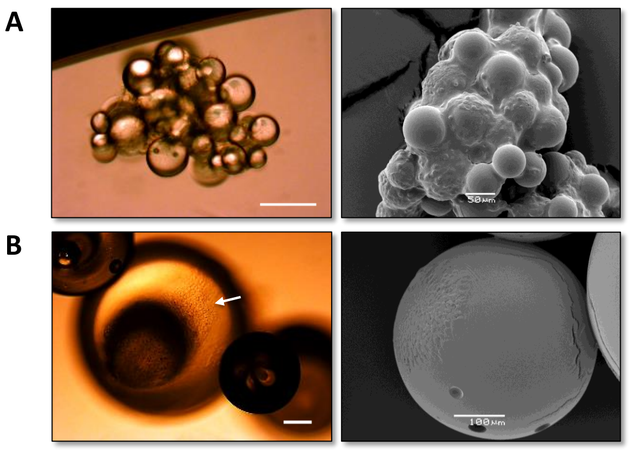


Figure 3. Bright field images (left, scale bars: 100 µm) and SEM images (right) of A) 3D cell-particle aggregates and B) cells-single particle constructs.

MP size greatly influence cell growth. Cells seeded on 50-100 µm MP formed large 3D cell-particle aggregates (Figure 3A) while cells seeded on large MP (200-300 µm) grew as pseudo 2D cultures on individual particles (Figure 3B).

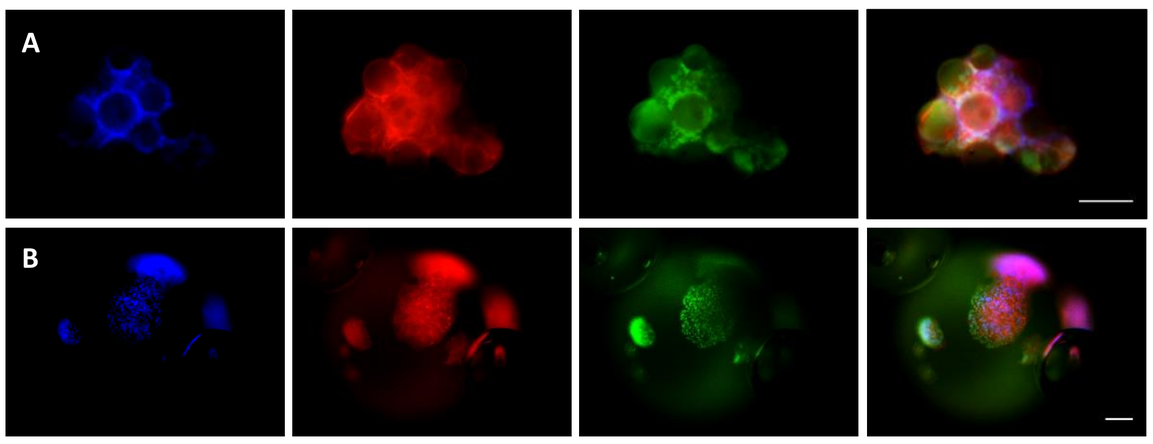


Figure 4. Fluorescence microscope images of A) 3D cell-particle aggregates and B) cells-single particle constructs. Scale bars: 100 µm. Cell nucleus stained with DAPI (blue) and Oct4 (stem cell marker, green). Cell cytoplasm stained with Phalloidin (red). Final images merged.

CONCLUSIONS

- PLA MP have been shown to support hESC adhesion and growth.
- Particle surface chemistry greatly impacted cell behaviour. However, treatments must be carefully examined in order to preserve particle morphology.
- Particle size also proved to be fundamental in promoting 2D or 3D cell growth.
- Chemistries which have previously demonstrated significant capabilities in modulating cell differentiation will be incorporated onto these surfaces to further extend this research.

REFERENCES

[1] H.V Unadkat et al. (2011) *PNAS* **108:**16565-70
[2] A.K Patel et al (2015) *Biomaterials* **61**:257-65
[3] A.J Engler et al (2006) *Cell* **126**:677-89.

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