Development of sol-gel derived hydrogels for bio-fabrication

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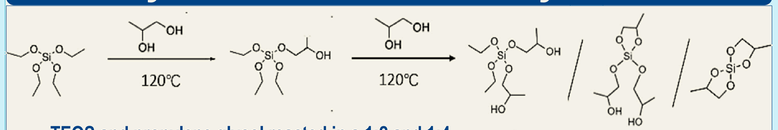
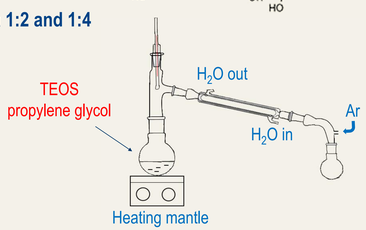
Introduction

The first bioactive glass, Bioglass®, was invented by Larry Hench in 1969. Bioactive glasses are clinically used as bone grafts where, they are known to form strong bonds to bone and release silicon species and Ca^{2+} ions, which can stimulate bone regeneration [1]. "Soft chemistry" sol-gel processing has enabled the development of organic-inorganic hybrid materials for tissue regeneration[2]. Hybrids have intermolecular mixing of the organic and inorganic moieties leading to substantial benefits over glasses or traditional composites. However, the harmful chemical used and alcohols evolved during sol-gel processing, so hybrids have to be dried before implantation or cell culture. Therefore, to facilitate bio-fabrication where cells, biomolecules and hydrogels are geometrically arranged in a desired 3D structure, using a bioactive glass or organic-inorganic hybrid as the hydrogel, new sol-gel materials and processing conditions need to be developed [3]. In this project, tetraethyl orthosilicate (TEOS) was transesterified with propylene glycol to remove the ethoxy groups, which are a source of ethanol which is harmful to cells. The propylene glycol-modified silane was then used to produce a new hydrogel for bio-fabrication.

Polyol-modified silanes synthesis



TEOS and propylene glycol reacted in a 1:2 and 1:4 molar ratio at 120 ℃



Ethanol (by-product) was continuously removed by distillation

Hydrogels

Hydrogel formation in water and Dulbecco's Modified Eagle Medium (DMEM) at different PGMS : water / DMEM molar ratios.

Medium:
Water
DMEM
Water+0.0024 M mol/L Ca^{2+}
Water+0.0024 M mol/L Mg^{2+}
Water+0.0024 M mol/L Na^{+}

Propylene glycol modified silanes (PGMS)

At ratio
(1:24, 1:48, 1:96, 1:144, 1:192)

Hydrogels

PGMS hydrogels with DMEM

Cell viability

PG_4MS hydrogels were prepared in 1:144 ratio using McCoys media. Osteoblast (SAOS-2) cells were encapsulated in hydrogels at density of 1 × 10^6 per mL of hydrogel by mixing 200 µL of a stock cell solution containing 1 × 10^6 cells with 1 mL PGMS solution. From this, 200 µL of mixture was pipetted into petri dishes. A two-colour fluorescence assay (LIVE/DEAD Assay) was employed to determine the cell viability in hydrogels at 24, 48 and 72 hours. The hydrogels containing the staining agent were left to stain for 20 minutes at room temperature. Zeiss LSM 700 confocal microscope was used for image acquisition. Cell viability was calculated as (number of green-stained cells/number of total cells) × 100%.

Results

Precursor synthesis

PGMS with 1:4 ratio of TEOS to propylene glycol: PG_4MS

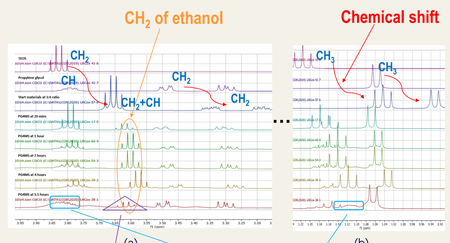


Figure 1. The expansion of ^{1}H-NMR spectrum; a) ^{1}H-NMR spectrum of TEOS, propylene glycol and PG_4MS at 3.20-3.95 ppm; b) ^{1}H-NMR spectrum of TEOS, propylene glycol and PG_4MS at 0.92-1.22 ppm

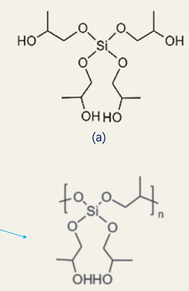


Figure 2. The chemical structure of PG_4MS Structure (a) and (b) (polymer)

PGMS with 1:2 ratio of TEOS to propylene glycol: PG_2MS

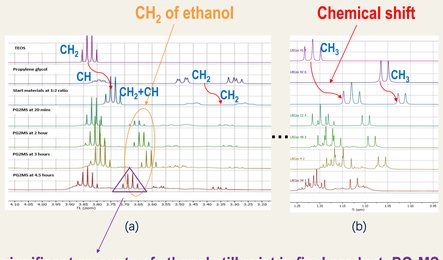


Figure 3. The expansion of ^{1}H-NMR spectrum; a) ^{1}H-NMR spectrum of TEOS, propylene glycol and PG_2MS at 3.20-4.10 ppm; b) ^{1}H-NMR spectrum of TEOS, propylene glycol and PG_2MS at 0.95-1.25 ppm

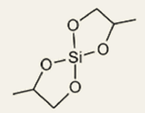


Figure 4. The chemical structure of PG_2MS

Hydrogels

ratio (medium to PGMS) ↑ the time to form hydrogel ↑

gel speed in DMEM >> gel speed in water

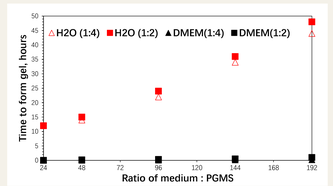


Figure 5. The time to gel of PGMS with H_2O / DMEM at different ratios

metal ions can decrease the time to form hydrogels

Ca^{2+} > Mg^{2+} > Na^{+}

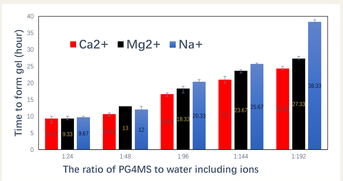


Figure 6. The time to gel of PG_4MS with H_2O including Ca^{2+}, Mg^{2+} or Na^{+}

Cell viability

not cytotoxic to the cells

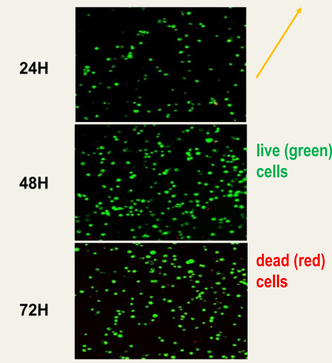


Figure 7. Confocal laser microscope images of osteoblasts encapsulated in PGMS for 24, 48 and 72 hours

The viability was ≥ 70% over culture time up to 72 hours

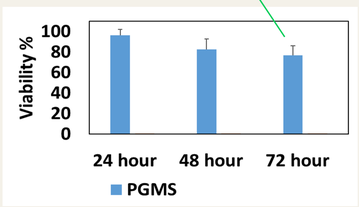


Figure 8. % Viability percentages of encapsulated osteoblasts in hydrogels at 24, 48 and 72 hours

Conclusions and Further Plans

In this project, a silane precursor, PGMS, for sol-gel processing into hydrogels was synthesised by transesterification of TEOS with propylene glycol. The synthesised precursor formed hydrogels which are suitable for cell encapsulation.
In the future, the hydrogel will be processed by 3D bio-fabrication, electrospinning and 3D printing to investigate its possible application in bone graft and its mechanical properties.

References

【1】Jones, J. (2013). Review of bioactive glass: From Hench to hybrids. Acta Biomaterialia, 9(1), pp.4457-4486.

【2】Poologasundarampillai, G., Ionescu, C., Tsigkou, O., Murugesan, M., Hill, R., Stevens, M., Hanna, J., Smith, M. and Jones, J. (2010). Synthesis of bioactive class II poly(γ-glutamic acid)/silica hybrids for bone regeneration. Journal of Materials Chemistry, 20(40), p.8952.

【3】 Vueva, Y., Connell, L., Chayanun, S., Wang, D., McPhail, D., Romer, F., Hanna, J. and Jones, J. (2018). Silica/alginate hybrid biomaterials and assessment of their covalent coupling. Applied Materials Today, 11, pp.1-12.