Improving Cell Survival and Proliferation in Novel Degradable PVA-Tyramine Hydrogels

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INTRODUCTION: Biosynthetic hydrogels, composed of synthetic and natural polymers, are structurally similar to the extracellular matrix and can potentially be used as tissue engineering matrices. However, long term usage of these gels requires the natural polymers, such as proteins, to be covalently bound into the network. Previous strategies involved chemically modifying the protein to introduce crosslinkable groups that can be co-polymerised with the synthetic polymers. However, chemical modification can cause protein denaturation or loss of biological activity. We have previously shown that a photo-crosslinked degradable synthetic hydrogel, made from poly(vinyl alcohol) (PVA) conjugated with pendent tyramine groups (PVA-Tyr), allowed for the successful covalent incorporation of unmodified proteins[1]. However, preliminary 3D cell encapsulation studies indicated that this photo-crosslinking process was detrimental to cells, possibly due to the production of radical species. Therefore, this study aimed to promote cell viability during the photo-encapsulation process by incorporating two anti-oxidative proteins, sericin and gelatin, into the PVA-Tyr hydrogels. The ability to successfully incorporate these proteins into the biosynthetic gels, and the effect of their incorporation on cell viability and growth in 3D gels was tested.

Methods: PVA was reacted with succinic anhydride and triethylamine for 24hr to form carboxylated PVA. This was further reacted with tyramine using carboxyl/amine coupling chemistry. Hydrogels (20wt%) were fabricated with 2mM tris(2,2'-bipyridyl) ruthenium (II) chloride hexahydrate and 20mM sodium persulphate under visible light (15mW/cm2) for 3 min. Gels were 20% PVA or 19% PVA with either 1% Sericin (19/1 PVA-Tyr/S) or Gelatin (19/1 PVA-Tyr/G), or 1% of both proteins (18/1/1 PVA-Tyr/S/G). For cell encapsulation studies, L929 murine dermal fibroblasts were encapsulated at a concentration of $1x10^6$ cells/ml. The gels were incubated in media then stained with a live/dead assay at various time points (Calcein-AM/ Propidium Iodide). An ATP assay was also conducted to evaluate the metabolic activity of the encapsulated cells.

Results: Pure PVA-Tyr gels were previously reported to exhibit a linear degradation profile over 3 weeks[1]. It was speculated that *if* the incorporated sericin and gelatin were able to mitigate the effects of the radicals generated during the photo-crosslinking process, it could also affect the quality of the gels produced. However, mass loss and swelling studies showed no significant difference between pure PVA-Tyr gels and those that contained proteins. The encapsulation of cells was where the real differences between these gels were observed. Pure PVA-Tyr gels did not support cell survival (Fig 1), most likely due to the generation of sulphate radicals during the polymerisation. As gelatin and sericin are both known to be anti-oxidative due to the abundance of hydroxyl amino acids (serine and threonine)[2], it was hypothesised that incorporation of these proteins could protect the cells. The addition of sericin resulted in fibroblasts surviving the encapsulation process. However gelatin did not have the same effect, unless it was combined with sericin. The real benefit of gelatin was seen at later time points. The majority of cells in 19/1 PVA-Tyr/S gels were able to form cell aggregates after 14 days of incubation. However, when both sericin and gelatin were combined in the gels, it was clearly seen that the cells not only formed aggregates, but were also able to spread and form cell-cell contacts at 14 days. This result was also confirmed by the ATP assay where only 18/1/1 PVA-Tyr/S/G samples showed an increase in ATP levels from 1 to 14 days (Fig 2). Immunocytochemistry results also showed that the cells were able to secrete collagen IV and laminin in the gel. Further encapsulation of other cell types (e.g., Schwann cells, astrocytes, glial cells) into this system have also showed promising results and are the focus of future work.

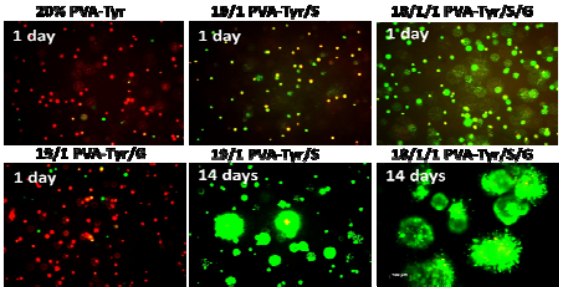

Fig 1: Live-dead staining of L929 cells in PVA-Tyr and PVA-Tyr/protein gels. Live=Green; Dead=Red.

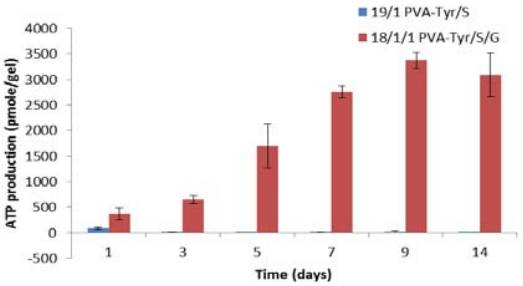

Fig 2: ATP production of L929 cells in PVA-Tyr/protein gels.

Conclusions: This study showed that incorporation of anti-oxidative proteins into PVA-Tyr gels successfully promoted the survival of encapsulated cells. We also demonstrated that sericin had a higher anti-oxidative effect than gelatin, but was not sufficient to promote cell spreading. The combination of both proteins was required to improve cellular activity in this 3D encapsulation system, which will be the focus of our future studies.

References:

1.Lim KS, et al. Biomaterials. 2013;**34**(29): 7097-7105.
2.Dash R, et al. BMB reports. 2008;**41**: 236.