Protein and Polyphenols Contaminants of Sodium Alginates and Their Effect on Viability of Mesenchymal Stem Cells Derived from Human Umbilical Cord

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Introduction: Alginic acid is a linear polysaccharide that mainly consists of two building blocks of mannuronic (M) and guluronic (G) acid residues. This biomaterial is frequently employed in variety of biomedical and pharmaceutical applications such as dental impressions, wound dressing and controlled drug delivery[1]. Due to their ionogeling property (ionotropic gelation), excellent biocompatibility and pliability, alginate gels are particularly suitable as scaffolds for tissue engineering applications. Apart from biocompatibility and batch-to-batch reproducibility, a successful clinical trial for such applications requires the alginate material to be pure. Proteins and polyphenols are the most common impurities in the pharmaceutical grade alginate polymers[2], and the use of such polymers as a tissue engineering scaffold requires a comprehensive evaluation of the contaminants and their role in cell viability.

Objective of the study: To determine the amount and the effect of proteins and polyphenols of pharmaceutical grade sodium alginates on cell viability of Human Umbilical Cord Cells (hUMSCs).

Methods: Two high G (α-L-guluronate) content alginates (Protanal LF200S and Protanal LFR5/60), as well as two low G content alginates (Protanal LF10/60LS and Protanal LF 240D) (FMC Biopolymers, Philadelphia, PA) were used in this study. Using a syringe fitted with a 25G needle, alginate beads were prepared by extruding a 2wt% sodium alginate solution into an aqueous 100mM $CaCl_2$ solution. The standard colorimetric MTT assay was conducted to investigate the cell viability of hUMSCs in the presence of alginate beads. The amount of protein content of alginates was determined by BCA protein assay (Thermo-Fisher Scientific Rockford, IL). The total phenol content of alginate was estimated utilizing Folin Ciocalteu (FC) reagent method using Quercetin as a standard[3].

Results: Compared to the control, alginate treatment resulted in an increased cell proliferation. Between low and high G content alginates, the latter supported cell growth significantly (mean ±SD, n=4; $p<0.05$). In the low G content group, the protein content was found in the range of 4.1-6.8mg/g of alginate. On the other hand, high G content alginates showed a protein content in the range of 3.4-7.5mg/g of alginate. The polyphenols concentration was found in the range of 1.0-5.1mg/g and 1.9-2.4mg/g of alginate for low and high G content alginates respectively.

	G/M%	P[1] PP	[2]	% Viability	
				30mg[3]	60mg
I	35-45/ 55-65	4.087	0.100	104.96	97.77
II	30-35/ 65-70	6.779	0.513	97.02	98.81
III	65-75/ 25-35	3.370	0.190	101.02	113.01
IV	65-75/ 25-35	7.473	0.240	116.68	116.23

1: Protein content, mg/gram dry weight of alginate; average of two experiments
2: Polyphenols content, mg/gram dry weight of alginate, average of three experiments.
3: Amount of alginate bead
I: Protanal LF10/60LS
II: Protanal LF240D
III: Protanal LF200S
IV: Protanal LFR5/60

Conclusions: The results showed that the high guluronic acid content in the alginates improved the viability of the hUMScs. The two major contaminants i.e. protein and polyphenols in the pharmaceutical grade alginates apparently did not influence the viability of the hUMSCs.

References:

1) H Omidian et al., Martin's Physical Pharmacy and Pharmaceutical Sciences 6th edition, Lippincott Williams& Wilkinks p 511
2) G Orive et al., Biomaterials 2002, 23 (3825-3831)
3) YT Tung et al., Bioresource Technol 2007, 98 (1120-1123)