Developing Permissive Hydrogels with Physiological Osmolarity for 3D Cell Growth

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Statement of Purpose: Injectable hydrogel becomes a popular research topic in the last decade, with the advantage of less wound in an easier accurate-targeting surgical operation and the ability to fit into irregular shapes. Chenite et al found that chitosan solution remains stable at room temperature when glycerol phosphate disodium salt (GlyPh) is added to bring pH up to close to physiology value, and gelling happens at physiology temperature 37 °C. [1] However, to obtain effective gelling at 37 °C in ca 30 minutes, very high content of GlyPh is required and hence high osmolarity is unavoidable, as GlyPh contributes the most to osmolarity. It is a common sense that 3D cell culture should avoid large deviation of pH and osmolarity from physiology values. The exact tolerance extent of such deviation is a critical factor for obtaining an optimum balance between different properties, but it was seldom paid special attention. Here systematic study was taken in 3D cell culture to study the effects of osmolarity in a model gel system of gelatin/ chitosan.

Methods: In a 5ml vial, 0.1 ml 1.6% g/ml solution of gelatin in water was mixed with 0.4ml 1.7 % g/ml solution of chitosan in 0.07 M acetic acid, and then desired amount of water and 0.088 g/ml NaCl in water (Table 1) were added to adjust the total volume to 1ml. NIH/3T3 fibroblasts cells were encapsulated in gels, above which culture medium was added.

Table 1. Formulas of hydrogels with different osmolarities

Formula #		*1*	*2*	*3*	*4*	*5*	*6*
Diluting water (ml)		0.5	0.475	0.45	0.25	0.5	0.5
NaCl solution (ml)		-	0.025	0.05	0.25	-	-
GlyPh solution (ul)		40	40	40	40	60	80
pH		6.91	6.90	6.95	6.82	7.06	7.20
Osmolarity (mM/kg H_2O)	Measured	210	276	355	862	302	383
	Theoretic	252	331	404	982	365	467
	Coefficient	0.84	0.83	0.88	0.88	0.83	0.82

Results: When osmolarity was increased above physiologic value, cell morphology turned gradually shrunk and round, and cell viability was reduced. Significant change occured above osmolarity of ca 650 mM/kg H_2O.

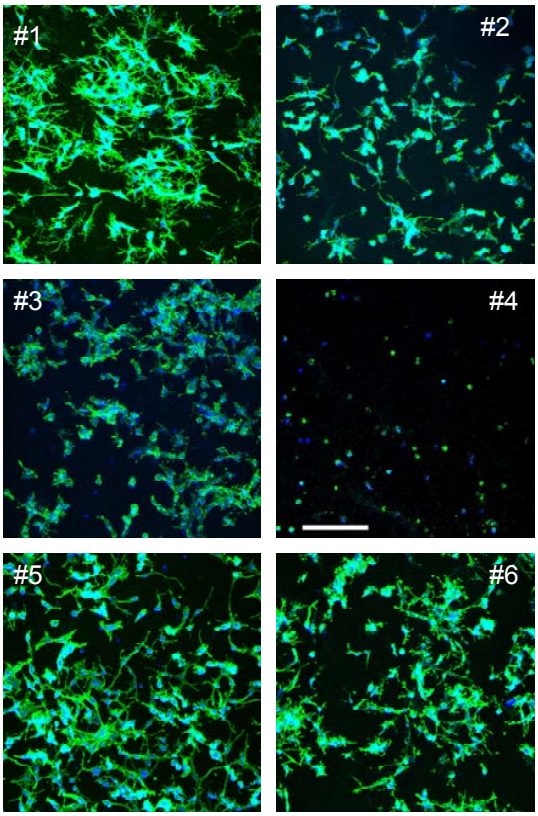

Fig 1. The maximum overlapping laser confocal images of fibroblasts 3D encapsulated (4.3×10^5 ml^{-1}) for 3 days in gels of recipes in Table 1. Actin and nucleus were stained in green and blue fluorescence, respectively. Scale bar is 200μm.

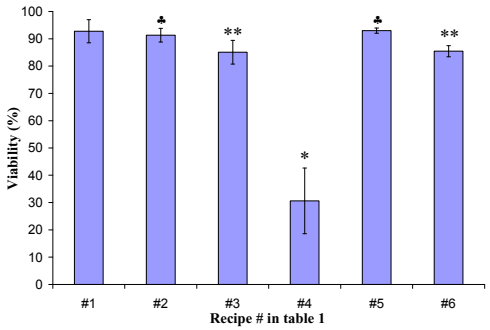

Fig 2. Cell viability based on live/dead stain of cells encapsulated for 3 days in gels of recipes in Table 1. The error bar was standard deviation. The statistical significance for equality of means comparing with recipe #1: * <0.001; ** <0.1; ♣>0.5.

Conclusions:
It is necessary to avoid high osmolarity when using chitosan-GlyPh hydrogel for cell encapsution.

References:
[1] A Chenite, et al. Biomaterials. 2000; 21: 2155-61.