

## The Use of Thermo-Sensitive Chitosan as an Injectable Carrier for Bone Tissue Engineering

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### INTRODUCTION

Due to its biocompatibility, biodegradation properties and potential angiogenic potential, the natural biopolymer chitosan has been used in the biomedical field. In combination with glycerol phosphate (GP – disodium salt), this cationic polyelectrolyte becomes thermosensitive in diluted acids and can undergo gelation around body temperature [1]. This property makes it promising for use in injectable tissue engineered bone, by acting as a delivery vehicle for cells and biomaterial microparticles.

In this study, we monitored the gelation time of 1% chitosan-HCl-GP solutions by adjusting the GP concentration (5-20%), and evaluated the cytotoxicity of the gels by monitoring the growth rate (proliferation) of goat bone marrow derived stem cells (gMSCs).

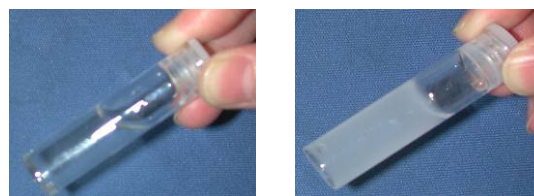
### MATERIALS AND METHODS

Chitosan (BMSI, China) with a nominal 90% deacetylation rate (DDA) was dissolved in HCl to form a 1% solution. Subsequently, 5%, 10%, 15% or 20% (w/v) glycerol phosphate (GP) was added drop-wise to form a uniform solution and the pH was measured. The gelation or polymer transition time of the solutions was measured by soaking them in a 37°C water bath.

Cytotoxicity of the gels was evaluated by an extraction test according to ISO10993-5. In short, the chitosan-GP gels (5-20% GP concentration) were immersed in normal culture medium (1 ml per 1.25 cm<sup>2</sup> gel surface area) for 24 hours at 37°C/5% CO<sub>2</sub>. The extraction fluid was then removed and placed onto a semi-confluent layer of gMSCs in 12-well plates. Cytotoxicity (cell proliferation) was measured after 24 and 48 hours using the Alamar blue assay. Results were compared to a negative- (cells grown in normal culture medium) and a positive control (cells grown in PVC extract medium).

### RESULTS

Before polymer transition (gelation), all chitosan-GP solutions had pH values around the physiological range (table 1). The gelation time of the various chitosan-GP solutions (with various GP concentrations) was time-dependent. All solutions could gel at 37°C, while those with more than 10% GP formed gels from seconds up to a few minutes (table 1). During the gelation process, the gels turned from transparent to opaque (figure 1).



a) 22°C

b) 37°C

**Figure 1.** Chitosan (1% w/v)-GP solution before gelation (a) and after gelation (b)

Cytotoxicity of chitosan-GP gels increased with increasing GP concentrations (table 1). Interestingly, extraction fluids from chitosan-GP with GP concentrations of up to 10% enhanced the proliferation of gMSCs 4 to 11-fold (table 1) as compared to the negative control. GP concentrations of 15% gave a growth inhibition to 65% of the control, while 20% GP was highly cytotoxic.

**Table 1.** Relation of GP concentration in chitosan-GP solutions to pH, gelation time, cell proliferation

GP Concentration (w/v)	5%	10%	15%	20%
pH before Gelation	7.0	7.3	7.5	7.6
Gelation Time at 37°C (min)	720	60	4	0.7
Cell proliferation compared to NC*	1100 %	400 %	65%	0%
Toxicity	none	none	mild	severe

\*NC= negative control.

### DISCUSSION & CONCLUSIONS

Chitosan with low GP concentrations (5-10%) is not cytotoxic but enhances the growth and proliferation of gMSCs compared to negative control. Nevertheless, long gelation times associated with these GP concentrations are a possible obstacle, which might be solved by adjusting the molecular weight, purification, or increasing the DDA of chitosan. These are the subjects of our future investigations.

### REFERENCES

1. Chenite et al. *Biomaterials* 21:2155-2161 (2000)

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