INTRODUCTION. To deliver and retain viable repair cells in a surgically prepared cartilage lesion, we previously developed an adhesive in situ-gelling cell carrier using cells suspended in a solution of hydroxyethyl cellulose (HEC) which was then mixed with chitosan-glyoxal phosphate (chitosan-GP/HEC) [1-3]. This gel supported cartilage matrix deposition by primary articular chondrocytes, and was retained in full-thickness chondral defects in living rabbits for 1 to 7 days [2]. Chitosan-GP/HEC hydrogels formed within 3 to 30 minutes using 2.5 to 5 mg/mL commercial-grade HEC, although medical-grade HEC failed to induce chitosan-GP gelation. Since commercial-grade HEC is routinely surface-treated with glyoxal to impede lumping [6], and glyoxal is known to covalently cross-link chitosan fibers [7], this led us to hypothesize that trace glyoxal present in commercial HEC was responsible for chitosan-GP/HEC hydrogel solidification. We studied the effect of HEC and glyoxal on gelation kinetics, gel shrinkage, and cell viability using several cell types. In a novel cartilage repair application, sheep mesoarthroplasty surgical defects were "grouted" with chitosan-GP/HEC hydrogel ex vivo and in vivo.

MATERIALS AND METHODS

Gelation tests. Medical-grade chitosan (BioSyntech, Laval, QC, Canada) was dissolved in dilute HCl, autoclave sterilized, and combined with filter-sterile disodium beta-glyceral phosphate (GP) to yield solutions containing 16 mg/mL chitosan (260,000 Da, 80%DDA), 135 mM GP, pH 4.10 in 0.05 M sodium HEC (Sigma-Aldrich, Oakville, ON, Canada) or glyoxal-free HEC (NF250; Anachemia, Montreal, QC, Canada) was dissolved at 5 to 25 mg/mL in phosphate-buffered saline or serum-free culture media and filter-sterilized. HEC NF250 surface-treated with glyoxal (HEC-glyoxal 1250 ppm) was generated in ethyl acetate followed by solvent evaporation. Manual gelation tests were performed on 4:1 mixtures of chitosan-GP to filter-sterile glyoxal and/or HEC.

Cell encapsulation. Pellets of 5 million cells were suspended in 200 µL cross-linker solution (HEC and/or glyoxal), and 150 µL of the cell suspension was vortex mixed with 600 µL of chitosan-GP solution in a sterile cryovial. The solution was poured into a 30 mm Petri dish or injected into a 30 mm Petri dish. Monolayer cells exposed to 1 mM glyoxal showed no loss in metabolic activity after 2 hours of exposure (Fig. 2A) while up to 50% of cells failed to survive encapsulation in chitosan-GP solutions cross-linked with 150 µM glyoxal (Fig. 2B). More viable cells were obtained at day=1 with commercial-grade HEC cross-linker (Fig. 2C). In ex vivo mosaic arthroplasty defects, chitosan-GP/HEC gels loaded with viable chondrocytes formed an adhesive osteochondral seal (Fig. 3A-D). In live animal defects however a blood interface formed between the mosaic arthroplasty defect base and the hydrogel carrier (Fig. 3E-F).

DISCUSSION. For clinical practice, an injectable hydrogel loaded with cells should optimally gel in situ within 5 to 10 minutes, and maintain maximal cell viability after gelation. Commercial HEC gelled chitosan-GP solutions within 5 to 10 minutes and maintained high viability, possibly due to the ability of chitosan-GP/HEC gels to form with lower glyoxal concentrations (Fig. 1A). Chitosan-GP/HEC gels formed an adhesive seal with ex vivo mosaic arthroplasty defects, while in live animal defects the gel adhered to bone, cartilage, and blood clot. These data show that chitosan-GP/HEC gels have promising features as injectable cell delivery vehicles for a variety of orthopedic applications.