INTRODUCTION

Marrow-stimulation techniques are commonly used by clinicians to repair articular cartilage lesions, but do not lead to consistent regeneration of hyaline cartilage. Chitosan is a biocompatible, biodegradable and non-toxic polysaccharide that has been shown to improve healing of connective tissues in several species. A cytocompatible chitosan-glycerol phosphate (C-GP) solution has been developed [1] and can be mixed with autologous blood to form a viscous C-GP/blood solution that adheres to tissue surfaces and solidifies. These implants are applied to marrow-stimulated chondral defects, where they gel in situ and improve cartilage healing. We have previously shown that application of C-GP/blood implants to marrow-stimulated chondral defects in rabbit [2] and sheep [3] leads to increased quantity and quality of cartilage repair tissue that is well attached to porous bone containing vascularized marrow spaces. Here, we have studied cell recruitment and repair tissue stages at early times post-surgically in a rabbit repair model to further understand the mechanisms by which these implants mediate cartilage repair. Our hypotheses were that C-GP/blood implants would i) increase recruitment of inflammatory and bone marrow-derived stromal cells, ii) increase vascularization of the microdrill holes and iii) modulate subchondral repair tissue development.

METHODS

The C-GP solutions used for this study consisted of 1.7% (w/v) chitosan (79.9% ± 2.8% degree of deacetylation) and 135mM disodium β-glycerolphosphate, pH = 8.60 ± 0.05, osmolality = 438±26 mOsm. Thirty-three skeletally mature NZW Rabbits underwent bilateral arthrotomies, with each trochlea receiving a 4x5 mm chondral only defects (~0.16 mm deep) further drilled with four 0.9 mm dia. microdrill holes (~4 mm deep). One knee per rabbit was treated by applying a C-GP/Blood implant over the defect. The implant consisted of the above C-GP solutions mixed (1:3 v:v) with fresh peripheral autologous blood. The contralateral control knee was left microdrilled. The rabbits were sacrificed at 1, 3, 7, 14, 21, 35 and 56 days post-treatment (n=3-8 for each time point). The rabbit femurs were fixed in 4% paraformaldehyde, decalcified in EDTA and cryosectioned. Volume density (Vv = cell volume per tissue volume) of neutrophils and bone marrow-derived stromal cells in microdrill holes was calculated on Safranin O/Fast Green stained sections by point counting in microscopic fields taken at 3 systematic positions in each of the 4 microdrill holes. Length density (Lv = length per tissue volume) of blood vessels in microdrill vessels was calculated on Gomori Trichrome stained sections by counting blood vessel cross-sections in microscopic fields also taken at 3 systematic positions in each of the 4 microdrill holes. Subchondral tissue formation was evaluated by immunostaining cryosections with anti-collagen type I (Sigma), anti-collagen type II (DSHB) and anti-Collagen Type X (Sigma), revealed with ABC-AP and AP Red Substrate kits (Vector).

RESULTS

![Figure 1: C-GP/blood implants (I) were delivered to microdrilled chondral defects in rabbit trochlea where they gelled in situ (c,d), while control knees were only microdrilled (a,b). Recruitment of neutrophils (N) and bone marrow-derived stromal cells (S) to control (e,f) and C-GP/blood implant (g,h) treated knees seen here at 1 day (e,g) postoperative in superficial chondral regions and at 7 days (f,h) postoperative deeper in the microdrill holes was observed and quantified using stereological point counting methods.](image)

![Figure 2: Volume density (Vv) of neutrophils (N) in microdrill holes was higher in treated knees up to 21 days post-treatment (a). Volume density (Vv) of bone marrow-derived stromal cells (S) was higher in microdrill holes of control knees at 7 days post-treatment but thereafter was greater in treated knees, from 14 to 56 days post-treatment (b). * p < 0.05.](image)

![Figure 3: C-GP/blood implants increased vascularization (V) in the microdrill holes of treated knees at 14 days (c,d). Length density (Lv) of blood vessels was higher in microdrill holes of treated knees at 14, 21 and 35 days post-treatment (e). * p < 0.05](image)

![Figure 4: C-GP/blood implants increased subchondral bone remodeling (see asterisks in b versus a) and delayed hypertrophic cartilage (C) formation in the microdrill holes of treated knees (d) at 14 days post-treatment.](image)

DISCUSSION

C-GP/blood implants were delivered to microdrilled trochlea defects in adult rabbits where they solidified in situ (Fig 1). Increased recruitment of neutrophils to the chondral area and microdrill holes of treated knees compared to controls was observed (Figs 1a and 2a). C-GP/Blood implants increased vascularization after day 7 (Fig 3), increased recruitment of bone marrow-derived stromal cells after day 14 (Fig 2), and delayed formation of hypertrophic subchondral cartilage (Fig 4) in the microdrill holes of treated knees. Furthermore, the implants promoted subchondral bone remodeling, including bone resorption and intramembranous new woven bone (NWB) formation in areas adjacent to the microdrill holes (Fig 4). Vascular-mediated supply of diffusible factors, progenitor cells, oxygen and nutrients may be partly responsible for improved cartilage repair with C-GP/blood implants found in previous studies. For example, close juxtaposition of NWB and newly formed blood vessels was observed in microdrill holes below C-GP/blood implants. Angiogenic signals induced by the implants thus appear to be involved in generating a porous subchondral bone structure with well vascularized marrow cavities. The presence of this type of porous and vascularized subchondral bone may be essential for effective cartilage repair in methods that rely on bone marrow mediated cartilage regeneration.

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REFERENCES


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