INTRODUCTION:
Subchondral drilling initiates a cartilage repair response involving formation of chondrogenic foci in the subchondral compartment. The purpose of this study was to structurally characterize these sites of chondrogenesis and to investigate the effects of chitosan-glycerol phosphate/blood implants on their formation.

METHODS:
Thirty-two 8-15 month old New Zealand White rabbits received bilateral cartilage defects bearing four subchondral drill holes. One knee per rabbit was treated by solidifying a chitosan-GP/blood implant over the defect. After 1 to 56 days of repair, chondrogenic foci were characterized by histostaining and immunostaining. Cell morphology and distribution of glycosaminoglycan and collagen type II, I and X were characterized. Chondrogenic markers and proliferation were identified with Patched and Ki-67. Collagen fiber orientation was characterized by polarized light microscopy.

RESULTS:
Chondrogenic foci were categorized as nascent (< 0.1 mm³, homogenous, GAG and col II positive), mature (Fig 1, larger, GAG and col II positive, stratified cell morphology) or resorbing (extensive vascular invasion and endochondral bone formation). In the mature foci, glycosaminoglycan and collagen type II were present throughout the foci while the upper zone expressed collagen type I and the lower zone collagen type X (Fig 1). Mature chondrogenic foci had a stratified structure with flatter cells closer to the articular surface, and round or hypertrophic chondrocytes deeper in the drill holes that showed signs of calcification after 2 weeks of repair in control defects (Fig 1). Markers for pre-hypertrophic chondrocytes (Patched) and for proliferation (Ki-67) were detected within foci (Results not shown). Some cells displayed a columnar arrangement where collagen was vertically oriented (Results not shown). Treatment with chitosan-glycerol phosphate/blood implants led to three important and statistically significant modifications of chondrogenic foci: 1) the initial appearance of foci occurred 1 to 3 weeks later (Fig 2), 2) more nascent and mature foci were produced than resorbing (Fig 2 and 3) foci were closer to the articular surface (Fig 3).

DISCUSSION:
Chondrogenic foci arising in cartilage repair from subchondral bone marrow stimulation bear some structural similarities to growth plate and developing articular cartilage in terms of cell morphology, the patterning of collagens type I, II, X and the signals that regulate chondrocyte differentiation, maturation and proliferation, but they display a less stratified structure and lower degree of organization. The temporal and spatial formation of chondrogenic foci can be modulated by cartilage repair therapies. We have previously shown that applying chitosan-GP/blood implants to marrow-stimulated cartilage defects improves the hyaline quality of the cartilage repair tissue. Here we show that this improved outcome is partly related to a delayed appearance of foci towards the articular surface and a reduction of the appearance of resorbing foci. Cartilage repair therapies that produce a coordinated induction of chondrogenic foci and controlled appositional growth closer towards the articular surface could improve functional outcomes.

REFERENCES:
2. Slave M et al. 2006; in Operative Techniques in Orthopaedics.