ABSTRACT: Bleeding has been identified as an initiating event in surgical cartilage repair techniques such as microfracture and drilling [1,2]. We have recently shown that in situ solidification of blood clots stabilized with chitosan-glycerol phosphate (chitosan-GP/blood clots) over microfracture defects improved fill and hyaline repair in an ovine microfracture repair model [3]. The beneficial effect of chitosan on cartilage repair in this large-animal study appeared to be linked to repair of bone damaged by microfracture. To study the processes underlying these repair responses, we have developed a bilateral rabbit knee microfracture cartilage repair model consisting of rectangular trochlear defects with multiple small drill holes [4]. One bleeding defect is left to heal as a control, while the other is additionally treated with a chitosan-GP/blood implant. This rabbit cartilage repair study (n=16) was used to test the hypothesis that chitosan-GP stabilized blood clots could increase hyaline repair both over the drill holes and between the drill holes. Defects from 3 additional rabbits were analyzed at 1 day to characterize differences were evaluated using the Mann Whitney U test, t-test, and multivariate correlation procedures (Statistica 6.0).

RESULTS: An animal-specific ease or resistance to debridement of calcified layer was seen, since both bilateral defects between the drill holes were almost completely covered with a partial calcified cartilage layer, while other bilateral defects contained almost no residual calcified cartilage, yielding an average 40% cross-sectional area distribution of calcification remaining in initial defects (Table 1). When calcified cartilage persisted after 8 weeks of repair in bilateral defects of a rabbit, control defects could be completely devoid of repair tissue between the drill holes (Fig. 1, upper panels), while treated defects contained tufts of hyaline repair tissue directly associated with porous bone and a concomitant resorption of the calcified layer between the holes (Fig. 1, lower right panels). More repair tissue covered and was integrated with subchondral bone between drill holes in defects which were treated with chitosan-GP/blood (p<0.005, Table 1).

METHODS: Ultrapure chitosan was obtained from BioSyntech (free base form Laval, QC, Canada, 79% deacetylated). Sterile chitosan HCl solutions were combined with disodium beta-glycerol phosphate (GP, Tissue Culture grade, Sigma, St. Louis, MO) to yield a solution with 1.6% ± 0.1% w/v chitosan with 135 mM GP (liquid C-GP, also called BST-CarGel®), pH 6.7 – 6.8, osmolality 250 – 1000 mOsm. All protocols involving animals were approved by institutional animal care committees. Thirty eight defects were created in the knees of 19 skeletally mature New Zealand White female rabbits (8 to 13 months old, 3.8 to 6.5 kg) via small arthrotomies. A 3.5 x 4.5 mm chondral defect was made in the center of each trochlea with a microsurgical knife into, but not beyond, the calcified layer, and then 4 mm diameter microholes were placed in each of the four corners of the defect model Pride stuck drilling [2]. Bleeding from the marrow holes in these adult animals was minimal, but was augmented by poking down the holes with a 26G½ needle. In each rabbit, one trochlear defect was allowed to fill with bone-derived blood, and the other was overlayed with ~25 µl of chitosan-GP/whole autologous blood at a 1:3 v/v ratio, which clotted in situ before closing the knee. Bilateral defects were allowed to repair for 1 day (n=3) or 8 weeks (n=16) after which dissected femora were decalcified and sectioned at the level of the proximal holes and between the drill holes. Analyses included: O’Driscoll scoring [5], histomorphometry, and stereology, the latter to assess subchondral bone repair quality correlates with subchondral bone porosity. Our collective results indicated that chitosan-GP/blood implants elicited significantly more hyaline repair above a significantly more porous subchondral bone. Repair tissue integration and hyaline quality between the drill holes and the subchondral bone correlated significantly with the presence of vascularized, porous bone directly below the repaired defect.

RESULTS: An animal-specific ease or resistance to debridement of calcified layer was seen, since both bilateral defects between the drill holes were almost completely covered with a partial calcified cartilage layer, while other bilateral defects contained almost no residual calcified cartilage, yielding an average 40% cross-sectional area distribution of calcification remaining in initial defects (Table 1). When calcified cartilage persisted after 8 weeks of repair in bilateral defects of a rabbit, control defects could be completely devoid of repair tissue between the drill holes (Fig. 1, upper panels), while treated defects contained tufts of hyaline repair tissue directly associated with porous bone and a concomitant resorption of the calcified layer between the holes (Fig. 1, lower right panels). More repair tissue covered and was integrated with subchondral bone between drill holes in defects which were treated with chitosan-GP/blood (p<0.005, Table 1).

METHODS: Ultrapure chitosan was obtained from BioSyntech (free base form Laval, QC, Canada, 79% deacetylated). Sterile chitosan HCl solutions were combined with disodium beta-glycerol phosphate (GP, Tissue Culture grade, Sigma, St. Louis, MO) to yield a solution with 1.6% ± 0.1% w/v chitosan with 135 mM GP (liquid C-GP, also called BST-CarGel®), pH 6.7 – 6.8, osmolality 250 – 1000 mOsm. All protocols involving animals were approved by institutional animal care committees. Thirty eight defects were created in the knees of 19 skeletally mature New Zealand White female rabbits (8 to 13 months old, 3.8 to 6.5 kg) via small arthrotomies. A 3.5 x 4.5 mm chondral defect was made in the center of each trochlea with a microsurgical knife into, but not beyond, the calcified layer, and then 4 mm diameter microholes were placed in each of the four corners of the defect model Pride stuck drilling [2]. Bleeding from the marrow holes in these adult animals was minimal, but was augmented by poking down the holes with a 26G½ needle. In each rabbit, one trochlear defect was allowed to fill with bone-derived blood, and the other was overlayed with ~25 µl of chitosan-GP/whole autologous blood at a 1:3 v/v ratio, which clotted in situ before closing the knee. Bilateral defects were allowed to repair for 1 day (n=3) or 8 weeks (n=16) after which dissected femora were decalcified and sectioned at the level of the proximal holes and between the drill holes. Analyses included: O’Driscoll scoring [5], histomorphometry, and stereology, the latter to assess subchondral bone repair quality correlates with subchondral bone porosity. Our collective results indicated that chitosan-GP/blood implants elicited significantly more hyaline repair above a significantly more porous subchondral bone. Repair tissue integration and hyaline quality between the drill holes and the subchondral bone correlated significantly with the presence of vascularized, porous bone directly below the repaired defect. A stereological analysis of the subchondral bone plate pore volume fraction showed that treatment with chitosan-GP/blood clots increased the porosity of the plate both over and between the drill holes (p<0.005, Vv, Table 1). The marrow cavities present in the pores of the subchondral bone plate were highly vascularized (Fig. 1, open arrowheads). Chitosan-GP/blood clots elicited the formation of a more hyaline repair tissue and higher O’Driscoll score both between (p<0.005) and over the drill holes (p<0.05, Table 1). Absolute differences between treated and untreated were more remarkable between the drill holes, where defects treated with chitosan-GP clot implants at 8 weeks contained less calcified cartilage, and more hyaline repair tissue integrated with the subchondral bone. A multivariate regression analysis of all 32 defects showed that integration correlated significantly with the absence of calcified cartilage, and that hyaline repair quality correlated significantly with subchondral bone porosity.

DISCUSSION: These data suggest that hyaline cartilage repair and firm integration of repair tissue to bone is promoted by proximity of chondral repair cells to a vasculature that is structurally protected by bone. The generation of a vascularized porous subchondral bone plate could mediate a supply of oxygen and nutrients, or other marrow-derived factors that stimulate chondrogenic proliferation, differentiation, and hyaline matrix production. These results further support the concept that cartilage repair outcome following marrow stimulation techniques is strongly influenced by bone repair and subchondral events. Application of chitosan-GP/blood implants to acute defects elicits the formation of a porous vascularized subchondral bone favoring the regeneration of hyaline articular cartilage.