REPAIR OF SHEEP LONG BONE CORTICAL DEFECTS WITH COLLOSS®, COLLOSS E®, OSSAPLAST® ORTHO, AND ILIAC CREST AUTOGRATF

INTRODUCTION:
COLLOSS® and COLLOSS® E (OSSACUR AG, Oberstenfeld-Germany), are bone void fillers consisting of lyophilized type I collagen and other bone proteins extracted from bovine and equine bone, respectively and purified by methods similar to those used by Urist for isolating bone morphogenetic proteins (1). COLLOSS® has been shown to induce ectopic bone in rats (2) and to be equivalent to autograft in spinal fusion cages in pigs (3). The objectives of this paper were to see if COLLOSS® and COLLOSS® E were equivalent to autologous iliac bone grafts and an osteoconductive sintered porous β tricalcium phosphate preparation, OSSAPLAST® ORTHO (OSSACUR AG, Oberstenfeld-Germany), in stimulating repair of cortical defects in sheep long bones, and to determine cellular and tissue mechanisms by which they stimulated repair of such defects.

METHODS:
Eight holes 5 mm in diameter were drilled through the medial cortex of the proximal and distal metaphyses of the right tibia and metatarsal bones of 14 skeletally mature Rambouillet x Columbian sheep using procedures approved by the Institutional Animal Care and Use Committee (protocol # 04-205A-01). Defects were filled with (A) fresh iliac crest autograft (1ml=1cm 3), (B) COLLOSS® (20 mg), (C) COLLOSS® E (20 mg), (D) a mixture of COLLOSS® E (20 mg) and OSSAPLAST® ORTHO (1 ml mixed with blood ), (E) OSSAPLAST® ORTHO alone (1 ml mixed with blood), or (F) left untreated. Defect sites in animals 1-6 and 7-12 were assigned treatments so that 6 defects were treated with A, D, E and F and, 12 with treatments B and C. Sites in animals 13, 14 were allocated to provide 2 treatments with A, D, E, F, and 4 treatments with B and C. Animals in each group were euthanized according to the guidelines set forth by the AVMA Panel on Euthanasia (4) after surgery, and defects recovered for analysis by micro-computed tomography and tissue histology and histomorphometry.

For Micro CT (SCANCO Medical AG, Basserdorf, Switzerland) each defect was scanned in planes perpendicular to its long axis (XZ slices) and reformatted into planes parallel to the long axis (XZ slices) at an in-plane resolution of 25 µm. Relative bone material volume was calculated as a percentage of the total defect volume. After Micro CT scanning defects were fixed in formalin, decalcified, bisected in an XZ plane, and 5 µm sections stained with H&E and Masson’s trichrome. Sections were evaluated qualitatively by normal and polarization microscopy to determine cellular and tissue mechanisms of bone repair, and by histomorphometry carried out with a Bioquant Nova system. Mean and median values of total repair, bone, marrow, and non-union areas expressed as mm² and as percentages of the total repair, defect, or combined internal and external callus areas were calculated for 4, 8, and 24 weeks after surgery. For 24 week defects, mean values of empty osteocytic lacunae and Haversian systems were calculated. Significance of differences among means and medians were evaluated by one-way ANOVA with post-hoc comparisons by the Tukey-Kramer method, and the Kruskall-Wallis non-parametric ANOVA with Multiple-comparison Z-tests, respectively. Differences in the incidence of 4 week defects with bone in the center of their central long axis, of 4, 8, and 24 week defects with total non-unions, and of bone-forming OSSAPLAST particles were analyzed by Chi-square statistics.

RESULTS:
Autografts produced appositional, COLLOSS® and COLLOSS® E, membranous, and OSSAPLAST® ORTHO, appositional and membranous bone. Different amounts and percentages of bone at 4 weeks reflected exogenous autograft and OSSAPLAST® ORTHO more than new bone growth. By 8 weeks bone areas increased in all but the autograft group and defects filled with COLLOSS® or COLLOSS® E + OSSAPLAST® ORTHO had more bone than other groups. Percentages of bone in defects did not differ. Defect union was more frequent in autograft and COLLOSS® E plus OSSAPLAST® ORTHO, than untreated defects.

At 24 weeks all treated, and no untreated defects showed complete union (Chi-square 17.0, P < 0.000004), validating the sheep cortical defect model as a surrogate critical size defect model. More Haversian canals and less dead bone indicated increased remodeling in defect walls filled with COLLOSS®, and COLLOSS® E.

DISCUSSION:
All treatments accelerated repair, osteoinductive fillers stimulated defect wall remodeling, and COLLOSS® and COLLOSS® E were more osteoinductive and stimulated defect wall remodeling more than autograft bone.

REFERENCE: