A Natural Granular Resorbable Scaffold for Bone Regeneration in Sheep

INTRODUCTION:
The possibility of isolating and expanding autologous mesenchymal stem cells (MSC) from a small sample of bone marrow and driving them towards an osteogenic phenotype has led to development of bioengineered bone constructs. In a previous study of a clinically relevant animal model it has been proved that standardized biohybrids engineered from Porites granular coralline scaffolds can be used to efficiently repair large bone defects. However, the quantity of bone formed in defects filled with biohybrids, varied among animals and bone union did not consistently occur. Several authors have emphasized that balanced scaffold resorption and new bone apposition must be obtained for bone healing with both natural and synthetic ceramic scaffolds. Most importantly, it is not yet understood whether the resorption of the scaffold itself is mandatory to trigger bone formation by cells. To address this question, bioengineered bone constructs made of Acropora coral which have less resorbability properties than Porites coral were implanted with or without MSC in a sheep metatarsal segmental critical size defect.

MATERIALS AND METHODS:
Animals: Nine two-year-old, female sheep were used for this study. All experimental procedures were performed according to the guidelines published by the European Committee for Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of Lariboisière Medical Faculty.

Scaffold: Acropora coral scaffolds (mean pore diameter of 500 µm; porosity of 12%) were available as cubes of 3x3x3 mm³.

MSC Isolation and expansion: Bone marrow was aspirated from the iliac crest of each sheep. Non-adherent cells were discarded during media change, after 3 days of culture. Medium was changed three times a week. When the cells were confluent, MSC were detached and were plated (passage 1) at the density of 3x10⁵ cells/cm². At 80-90% confluence MSC were detached and cryopreserved.

Cell loading of scaffolds: One week before implantation, cryopreserved cells were thawed and washed, 10⁤³ MSC were seeded per cube of Acropora. All the cell constructs were placed in an incubator for 7 days.

Surgical procedure: Osteoperiosteal segmental (25 mm) defects were made in the left metatarsal bone of 9 sheep, stabilized by plate fixation, and replaced with a PMMA spacer. The PMMA spacer was removed 6 weeks later, and the defect was filled either with Acropora coral scaffolds (Group1; n=4); or with Acropora coral scaffolds loaded with MSC (Group2; n=5).

Postoperative Management: Postoperative analgesia was provided by intravenous injections of meloxicam and tramadol administered 30 min before and after each surgical procedure and again 7 h after the procedure. The lower limb was fitted with a short cylindrical cast.

In-vivo and post-mortem evaluation: Standard radiographs were taken after each surgery and at the end of months 1, 2, 4 and 6. Immediately after euthanasia, casts were removed, and the metatarsal bone excised. Bone explants were collected for processing. Bone healing and scaffold resorption were assessed by micro-computed tomography (SkyScan™ 1171 microCT) and undecalciﬁed histology. The data were reported as mean ±SD and analyzed by Mann-Whitney test. Values p<0.05 were considered signiﬁcant.

RESULTS:
All animals recovered well and stable bone ﬁxation for all animals was obtained throughout the study. Muscle wastage was observed on the operated limb in all sheep as early as 3 weeks postoperatively, suggesting an abnormal weight bearing pattern but this did not differ between Group1 and Group2. By 1 month, radiographs showed resorption of the coral scaffold in all animals but this process was not complete and not the same in all animal. Six months after implantation, animals were sacriﬁced and each defect was assessed by micro-computed tomography and histology. No bone union was observed in Group1, in contrast to Group2 where bone continuity was observed.

DISCUSSION AND CONCLUSION:
In the present study, the osteogenic potential of MSC/Acropora coral constructs for repairing long segmental critical-sized bone defects in sheep was investigated. MSC were loaded onto Acropora coral scaffolds which are less resorbable coral than Porites coral used in the previous study. Most importantly, the findings showed that granules of MSC/coral constructs enhanced signiﬁcantly the rate of new bone formation. High amounts of newly formed bone in defects ﬁlled with Acropora/MSC (Group2) were signiﬁcantly higher from those ﬁlled with Acropora alone (Group1) (677±227 v.s. 1357±471 mm³) (Figure 1B).

Figure 1: Resorption rate (B) and volume of newly bone formed (A)

Whereas cell-free implant rarely displayed bone formation (mostly observed in the bony edges vicinity) with an abundant ﬁbrous tissue, a consistent bone formation was observed with MSC, even inner the defect at distance to bony edges (Figure 2). Although the results remained variable among animals, a fully bone regeneration was obtained in one animal of the Group2 (Figure 2).

Figure 2: Representative 3D tomomdenosimetric reconstruction of sheep belonging to Group1 (A) and Group2 (B), and histology of sections inner the defect of sheep belonging to Group1 (C) and Group2 (D). The histological stain was Stevenel Blue and von Gieson picrofuschin. (Original magnification: x4; red: bone; brown: coral; *: ﬁbrous tissue)

SIGNIFICANCE:
This study established clearly the beneﬁts of using Acropora scaffold loaded with MSC for long bone defect in sheep since this tissue construct allowed to obtained a fully bone regeneration. Further studies are needed to understand inter-individual variabilty.

REFERENCES: