A NEW, SEGMENTAL, CRITICAL-SIZE DEFECT MODEL FOR LONG BONES: A SHEEP STUDY


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Introduction
Reconstruction of large segmental bone defects can be achieved by a two-steps procedure using cancellous bone chips as described by Masquelet et al. In a first surgical procedure a PMMA-cement spacer is inserted into the defect. The presence of this foreign body triggers the formation of a pseudo-synovial membrane. After 8 weeks, the cement spacer is removed leaving a cavity surrounded by a membrane. This cavity is then filled with autologous cancellous (ACG) graft. Successful repairs of bone losses up to 25 cm long have been reported in human with this technique. It, however, requires large quantities of autograft and, sometimes successive surgeries need to be performed to achieve bone union. The discovery of growth factors and skeletal stem cells have opened new avenues for designing highly efficient bone substitutes. A key step in the development of these novel biomaterials is their in vivo assessment in clinically relevant animal models. In the present study, we set up a animal model that mimicked the surgical procedure described by Masquelet and explore the possibility of using skeletal stem cells loaded onto a coral scaffolds to repair such defects.

Materials and Methods
MSCs amplification: MSCs are expanded in 10% FCS with osteogenic supplements.

Surgical model: 20 adult sheep underwent a 2.5 cm long mid diaphyseal bone resection of the metatarsus that was filled with a cement spacer for 6 weeks. At that time cement was carefully removed through an incision made along the formed pseudo-synovial membrane and replaced according the following protocols:

Experimental groups
Control groups: Defects left empty (n=4) or filled either with coral cubes (n=5) or autologous cancellous bone chips (n=5) harvested at the iliac crest were implanted as controls. Coral+MSCs group: constructs are composed of MSCs loaded onto natural coral cubes 3mm (n=7).

Results
Establishment of the animal model

At explantation, all defects left empty (Fig 1 A, B) (4/4) demonstrated no repair. In contrast, defects filled with or autologous cancellous bone always healed (Fig. 1 C and D) (5/5). Immunohistochemistry study of the membrane showed that it was a well vascularized (fig 2 A) type 1 collagenous membrane which contained cells positive for CBFA1(Fig 2 B) and very few macrophages.

Preparation of the bone constructs
MSCs proliferate on the coral and were at confluence after 8-10 days (fig 3). They were distributed throughout the granules, with a concentration towards the peripheral areas. Cell viability was good at the time of implantation.

Radiographic analysis showed that filling bone defects with coral/MSCs bone constructs improved bone formation compared to defects left empty or filled with coral cubes alone. However, the osteogenic potential of coral/MSCs bone constructs was low when compared to autografts. Histological observations showed a complete disappearance of coral scaffold. Defects filled with a bone construct did not exhibit any patent clinical repair when compared to autograft (see fig 1 C, D).

Conclusions
In this study, we were able to develop an experimental defect model on weight bearing bone closing mimicking the clinical procedure. This model appears to be a critical size defect as defects left empty never healed. An inappropriate mechanical environment might led to delayed or non-union. This was not observed in this study when implanting autograft, the benchmark of bone reconstruction, which always healed. The use of large animals allow the creation of defects of clinically relevant volume. It is noteworthy that as observed in patients, a membrane surrounding the defect and forming a sealed cavity was formed. The creation of such a closed space is of particular relevance in tissue engineering strategies as it provides a natural barrier against the leakage of biological agents. In this study, it allows the localization of MSC-loaded coral cubes within the defect. Alternatively, it could maintain Bone Morphogenetic Proteins within the defect avoiding their leakage in extra-bony site. Interestingly, the immunochemical data suggest that the membrane was well vascularised and contained CBFA1 positive cells suggesting that the membrane could act as a local source of osteoprogenitor cells. Nevertheless, further investigations are needed to better understand the role of the membrane.

In this model, radiographic analysis showed that filling bone defects with coral/MSCs bone constructs improved bone formation compared to defects left empty or filled with coral cubes alone. However, the osteogenic potential of coral/MSCs bone constructs was low when compared to autografts. The use of a greater number of MSCs or of a less resorbable scaffold should be of interest to improve the osteogenicity of these constructs.

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