Tissue Engineering for Lumbar Spinal Fusion in Sheep

J. Becerra[1,2], MD Cuenca-López[1,2], S. Gómez[3], P. Zamora-Navas[4,2], E. Guerado[5,2], JA Andrades[1,2]

[1]Department of Cell Biology, Genetics and Physiology, Faculty of Sciences. University of Málaga, Málaga, Spain
[2]Networking Biomedical Research Center in Bioengineering, Biomaterials and Nanomedicine (Ciber-bbn)
[3]Department of Pathological Anatomy, Faculty of Medicine. University of Cádiz. Spain
[4]Service of Orthopaedic Surgery and Traumatology, V. Victoria University Hospital, Málaga, Spain
[5]Division of Orthopaedic Surgery and Traumatology, Hospital Costa del Sol. Marbella, Spain
becerra@uma.es

Posterior lumbar spinal fusion is nowadays a very standardized surgical technique worldwide. The intervention consists of two main steps, a firm fixation by a hardwork instrumentation to provide mechanical stability, and the addition of a biological substance for bone formation enhancement. Grafting enhances bone fusion, and therefore permanent stability, being considered bone autograft as the gold standard. The goals for bone-graft substitutes are to match fusion rates with autologous bone grafting techniques, while avoiding the morbidity of bone graft harvest and extending the quantity of available graft material. So far, there is a long list of bone graft substitutes [1], and most of them need a carrier to be administrated in the surgical field. Anyway, spinal fusion models are particularly unique, compared to other types of bone repair. Spinal fusion requires not to recreate original anatomy but the formation of a heterotopic bone bridge where usually bone does not exist. This may be the cause of the high clinical failure rate, above 35% [2]. Allograft as substitute/carrier is an important osteoinductive and osteoconductive agent but it has been especially claimed that it can provoke disease transmission and immunogenicity [3].

Since osteogenesis is exclusively conducted by bone cells, a very important strategy when dealing with bone substitutes, consists of addressing research projects u-sing osteogenic cells as bone marrow (BM) mesenchymal stem cells (MSCs). Two main lines have been maintained during the last years: molecular stimulation by a growth factor-mediation fashion (BMP-2 and BMP-7) [4,5], and transplantation of cells after amplification and committement [6]. BMPs have demonstrated good fusion rates but questions including high cost, high dose needed and some adverse effects, make them a non-definitive therapeutic tools [7]. Regarding cells, since several types of stem cells are susceptible to *in vitro* differentiate into multiple skeletal lineages, and are able to form bone, when using with the appropriate scaffold, tissue engineering looks like a good substitute for auto- and allograft in orthopedic surgery [8]. Nevertheless, clinical and animal experimental models research have had very important methodological burdens. On the one hand, most of laboratory work have been made in rodent and lagomorphs, species behaving far away, better than human as far as osteogenesis is concerned; further experimental model did not take into account mechanical situations as in human. Anyway, tissue engineering of bone, by combining osteogenic cells with osteoconductive scaffolds, has not yet yielded any clinically useful applications so far.

We have created an experimental model in a large animal model, the sheep, trying to reproduce what is made in human, a mechanical stabilization by a screwed transpedicular lumbar spinal instrumentation, together with the addition of BM committed MSCs adsorbed on hydroxyapatite (HA).

Surgical procedure. Spinal process from L2-L6, laminae, facet joints, and transverse processes were neatly denudated and prepared for arthrodesis. After that, stainless steel pedicular screws (Xia®, Stryker™) were introduced. Bone decortication was acted upon lateral aspect of articular and transverse processes until some bleeding bone could be seen.

Groups. A group of 20 sheep (female, 3-4 year-old, weighing 50-70 kg) received autograft into the right side (AUTO) and allograft on the left one (ALO). Another 20 sheep received HA with MSCs on the right side (HA+cls), and HA alone on the left one (HA) as control.

Cell product. Twenty milliliters of BM aspirates were harvested. The mononuclear cells fraction were plated at a density of $10x10^6$ cells per 100 mm plate in complete culture medium (DMEM, 10% FBS, 1% Penicillin, 0,5% Amphotericin, 1.25% Glutamine, and 1ng/ml FGF-2) and incubated at 37ºC, 95% humidity, and 5% CO_2. The medium was changed three times a week and the cells at passage 1 were cultured for three weeks. At the end of the culture period, the cells were incubated for 3 days in culture medium supplemented with dexametasone and β-glycerophosphate to help with the osteogenic differentiation.

Results have shown that, although autograft obtained better values for fusion than allograft, there was no statistical significance difference between autograft and allograft. Further, autograft and allograft accomplished a better bone formation rate than committed stem cells. Hydroxyapatite alone produced the worst results both in regard to the amount of bone formed as the rate of spinal fusion. Since *in vitro* and *in vivo* studies with small animal models have shown that this construct displayed a very strong osteogenic commitment, we aim to improve the implant in sheep, perhaps by increasing its, the number of cells per volume unit, and even introducing certain improvements to the scaffold.

References

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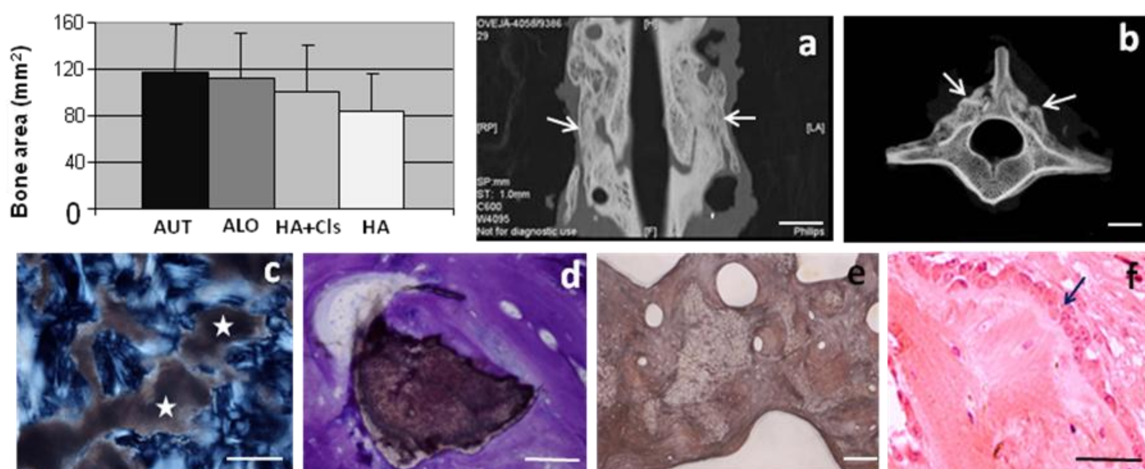

Graph showing bone area formed in the different types of implants. a) Coronal view (TAC) of the artrodesed segments showing fusion bridges of bone tissue between vertebral bodies (arrows). Bar, 1cm. . b) Axial view (TAC) where new bone in the area of the implant can be seen (arrows). Bar, 1cm. c) Tissue section under polarization microscopy showing HA (starts) surrounded by new bone. Bar, 250μm. d) HA fragment (brown color) in resorption surrounded by new bone type woven in an implant with MSCs (section stained with toluidine blue). Bar, 100μm. e) New bone in an implant with MSCs where woven bone types coexist with laminar and haversian types and resorption lacunae (Von Kossa stained). Bar, 200μm. f) Row of osteoblasts (arrow) in active synthesis of new bone (hematoxiline-eosine). Bar, 50μm