INTRODUCTION
Posterior lateral spinal fusion is a commonly performed and successful procedure for many orthopaedic indications. To create a bony bridge between two transverse processes, autologous bone grafts are commonly used, however several disadvantages like donor site pain persist. A promising alternative may come from tissue engineered (TE) bone, consisting of multipotent stromal cells (MSCs) seeded on porous ceramic scaffolds. This usually takes two interventions, bone marrow aspiration and the actual implantation operation. It would be preferable to perform a one step procedure with dynamic, as to use allogeneic MSCs. In the past years, it has become increasingly clear that allogeneic MSCs do not elicit a strong immune response in vitro and in vivo. Furthermore, the site of implantation has great consequences for the anticipated bone formation, i.e. at orthotopic locations bone-forming cells may be recruited from the underlying bone. Platelet leukocyte gel (PLG), acting as a source of growth factors, may further stimulate cell recruitment from underlying bone and cell proliferation. In this study we assessed the contribution of cells, either autologous or allogeneic, and PLG on bone formation in orthotopic and ectopic locations. We used an adapted version of a goat transverse process model to evaluate bone apposition in multiple conditions simultaneously. Furthermore we assessed the osteogeneity of hybrid constructs at an ectopic location.

MATERIALS AND METHODS
After approval by the animal care committee, bone marrow was aspirated from nine female adult Dutch milk goats four weeks before the implantation surgery. MSCs were isolated, culture expanded and cryopreserved after the 1st passage. At the day of surgery, autologous and allogeneic MSCs (from an earlier project, goats without a known direct family relationship) were taken to the operating room. Scaffolds were 12x8x3 mm (cassette implantation) and 7x7x7 mm (ectopic implantation) 75% macroporous ceramic blocks of a biphasic calcium phosphate (20%TCP/80%HA, Progentix, The Netherlands). Peroperatively whole blood was aspirated for plasma and PLG preparation. During surgery, the cells were resuspended in either platelet-leukocyte gel (PLG) or plasma and seeded on the scaffolds. Two scaffolds without cells served as control. The ectopic constructs were implanted in the paraspinal muscles of the goat. Polyethylene cassettes were mounted bilaterally on the decorticated transverse processes of the goat L1 vertebra (Fig. 1). Each cassette housed the BCP blocks (unseeded control, allogeneic, autologous MSCs) separated by Teflon sheets and was covered with either PLG or plasma. Fluorochrome markers were administered at week 3, 5 and 9 after implantation to assess bone growth dynamics. At 16 weeks, the implants retrieved and processed for histomorphometry. Statistical analysis was performed by repeated measurement analysis, followed by a Bonferroni post-hoc test (p<0.05).

RESULTS
Similar to previous work, clusters of lymphoid cells were present in the autologous MSC-seeded constructs ectopically. They did not contain neutrophils or promyelocytes, which can be interpreted as absence of an acute inflammation at the time of explantation. Lymphoid clusters were not present in the scaffolds seeded with autologous MSCs, control scaffolds, and in the cassette implants. Analysis of bone growth dynamics by fluorochrome incorporation in the cassettes, showed the week 3 label incorporated in the upper 3rd (most dorsal) of the scaffold when cells were seeded (in 10/36 implants) indicating early MSC-related osteogenesis, this was never seen in the non-cell seeded implants (0/18). In the lower (bone contacted), and middle part of the scaffold, cell seeding didn’t enhance bone formation, as no differences in fluorochrome incorporation were seen. Ectopically, in the cell-seeded constructs, bone formation started in 34/36 constructs before 3 weeks. This in contrast to the non-cell seeded constructs, where bone formation never started before this time point. Histology of the ectopic implants showed various amounts of bone apposition with significant differences between the cell-seeded and the control conditions, which were not observed in the cassettes (Fig. 2). When looking at bone contact% (BC%) in the ectopic implants, we found significantly higher bone apposition when autologous (p<0.03) and allogeneic (p<0.001) MSCs were seeded compared to the control scaffold. We did not observe a significant difference between any of these groups in the cassettes, while we did see an early positive effect in the upper 3rd when looking at the week 3 labels. No significant differences in bone formation were observed between autologous MSCs, plasma group 13.5±4.6 and PLG group 15.1±4.6, and autograft, plasma group 12.3±3.7 and PLG group 14.8±7.4 MSCs. Addition of PLG did not enhance bone formation (BC%) in the cassettes, but it did have a positive effect on the ectopic implants (p=0.035).

DISCUSSION AND CONCLUSION
After 16 weeks an additive effect on bone formation was seen ectopically, but not in the transverse process implants. We conclude that the material used has such good osteoinductive and -conductive characteristics, that the addition of cells does not influence the total bone content in the transverse process model, although it does accelerate early bone formation. Furthermore, to our knowledge we are the first to directly compare autologous and allogeneic MSCs at an orthotopic location for bone tissue engineering and found that both ectopically as well as orthotopically, no significant difference in bone formation between these cells was present, however equality was not shown. The lack of obvious difference in bone formation however is an important finding when considering the use of allogeneic cells as an off-the-shelf component in bone TE in goats.

In conclusion, our data indicate that the site of implantation and the material used greatly define the contribution of seeded MSCs in hybrid constructs; that no obvious differences were found between autologous and allogeneic MSCs with respect to bone formation, although tissue response was more pronounced at the ectopic location; that PLG is not effective at orthotopic MSC-seeded sites during a long follow-up period.

REFERENCES
1) Wilson CE et al., Biomaterials; 2006
2) Geuze RE et al., Tissue Engineering; 2008