INTRODUCTION

Tissue engineering of bone by combining bone marrow stromal cells (BMSC) with a porous osteoconductive scaffold is considered a promising alternative to autologous bone grafts. However, there are some important drawbacks. When using autologous BMSCs, there is a delay of at least two weeks due to culture expansion of the cells before re-implantation. Furthermore, expansion in culture makes it logistically difficult to prepare the scaffold/ cell construct coinciding with the operation of the patient. For this reason we compared two “off the shelf” procedures in an ectopic implantation model in the goat, namely direct seeding of: 1) peri-operatively harvested bone marrow; 2) culture expanded autologous bone marrow in allogeneic BMSCs and platelet gel (PG), a mixture of platelet rich plasma (PRP) and thrombin, on bone formation. It was previously reported that adding platelet concentrate to an autologous cancellous bone graft resulted in faster bone maturation and ultimately in more bone. This effect was attributed to the release of a wide range of growth factors, including TGF-β and PDGF, which are known to stimulate vascular ingrowth and cell migration. This led to the hypothesis that the use of platelet gel could also lead to a higher bone formation at an ectopic location for bone tissue engineering compared to the use of plasma to impregnate the scaffold.

The aim of this study was: (1) to investigate the effect of allogeneic cells compared to fresh bone marrow in a large animal model for ectopic bone tissue engineering; (2) to assess the potentially additional effect of platelet gel on ectopic bone tissue engineered constructs.

MATERIALS AND METHODS

After approval by the animal care committee, bone marrow was aspirated from the iliac wing of 10 mature 2-year-old Dutch milk goats for direct use. From the remaining bone marrow, the fraction of plastic adherent BMSCs was determined with a colony forming efficiency (CFU-E) assay. Furthermore, whole blood was aspirated from the external jugular vein for plasma and platelet gel preparation. Aliquots of cryopreserved allogeneic BMSCs, derived from an earlier goat study, were thawed and prepared for direct seeding. Scaffolds were 7x7x7 mm 75% macroporous ceramic blocks of a biphasic calcium phosphate (20%TCP/80%HA, Progentix, The Netherlands). During surgery, for each goat three scaffolds were impregnated with 300µl autologous plasma or platelet gel. Per condition one scaffold was left empty and served as a control, the second one was seeded with bone marrow (150x10^6 cells) and the third with allogeneic BMSCs (2,75x10^6 cells). During surgery, for the control scaffolds only the fluorescent label administered after five weeks was visible.

RESULTS

There were no surgical complications and all samples were retrieved after 9 weeks. Histology showed that all scaffolds were embedded well in surrounding tissue without any signs of inflammation. The colony forming efficiency was 1.6x 0.9 BMSCs per 10^6 nucleated cells. Bone was found in various amounts in all groups. The histomorphometric results are shown in figure 1. Bone was found in 100% (20/20) of the allo BMSCs-seeded scaffolds and in 85% (17/20) of the bone marrow-seeded scaffolds and finally in 35% (7/20) control scaffolds, which indicates the osteoconductivity of the material. Significantly more bone contact was found for the allo BMSCs/ plasma construct and near significance for the allo BMSCs/ PG construct versus their control scaffolds, 8,1% and 4,8% (p<0,0001 and p=0,054 respectively). No significant difference was found for the BM constructs, using either plasma (3,6%) or PG (1,5%) compared to their controls. Significantly more bone was found in the allo BMSCs/ plasma impregnated scaffolds (8,1%) compared to BM/ plasma (3,6%) constructs (p=0,03). Impregnation of the scaffold with either platelet gel or plasma did not differ significantly in bone contact% (p=0,05) for both treatment groups and controls. Fluorescent microscopy showed the three and five week’s label in the cell-seeded constructs indicating that bone mineralization started after two and before three weeks. For the control scaffolds only the fluorescent label administered after five weeks was visible.

DISCUSSION AND CONCLUSION

The current study demonstrates a clear difference in bone formation between the ‘off the shelf’ allo BMSCs/ plasma construct and the control scaffold. This trend is also shown in the allo BMSCs/ PG construct. This supports the notion that allogeneic BMSCs are immunoprivileged cells that in our study seemed to be responsible for the increased bone formation compared to the control scaffolds. We did not find a difference in bone contact between bone marrow seeded scaffolds compared to the controls, which can be partially explained from CFU-E results. There are only 58±30 BMSCs on the scaffolds seeded with BM compared to 2,75±10^6 BMSCs on the scaffolds seeded with allogeneic cells.

Unexpectedly, we did not find an additive osteoinductive or osteoconductive effect from the PG in this ectopic implantation model. Possibly the growth factor boost did not become available at the right time to the proper cells. Further research is required to clarify this.

REFERENCES

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