OPTIMIZATION OF BONE TISSUE ENGINEERING IN GOATS

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Introduction

Tissue engineering (TE) of autologous bone provides us with a promising alternative for autologous bone grafts. The method most extensively investigated is the combination of bone marrow derived stromal cells (BMSCS) with a porous ceramic scaffold. Since the early nineties, this concept has demonstrated to be successful in rodents both ectopic and orthotopically. However, upscaling this knowledge to larger mammals has been challenging, and only few reports have actually demonstrated ectopic or orthotopic bone formation in larger mammals, with limited “control” observations.1,2 Therefore we demonstrated successful bone tissue engineering in an ectopic goat model.1 The advantage of an ectopic model is that it allows fundamental research without the disturbance of osteoconduction as present orthotopically. Furthermore many conditions can be compared within one animal. At present, many strategies for bone tissue engineering can be found in literature. For instance, undifferentiated “stem” cells can be combined under serum free conditions with a scaffold and implanted within several hours.3 Others culture the constructs for one or more weeks before implantation to allow for differentiation of the cells and extracellular matrix (ECM) formation.4 Differences between these strategies can be deduced to, (a) the presence of an ECM at implantation, (b) the differentiation status of the cells, and (c) the presence of potentially immunologic serum proteins. In order to optimize our bone tissue engineering approach, we investigated these parameters in two calcium phosphate scaffolds in our goat model.

Materials and Methods

After approval by the animal care committee, bone marrow aspirates were taken from eight 2-year old Dutch milk goats. The BMSCS were culture expanded in culture medium with 15% fetal bovine serum (FBS) of a selected lot.4 When the cells had grown confluent these were detached and replated at 5000 cells per cm2. After another passage the cells were divided over different treatment groups. Two scaffolds types were used in the current experiment: HA70/800 - hydroxyapatite (HA) with a 70% interconnected porosity and an average poresize of 800µm, produced with spray dried HA powder which resulted in a marked microporosity (CAM Implants, the Netherlands); HA60/400 - a 60% porous, interconnected HA with an average poresize of 400µm, produced from commercial HA powder (Merck, the Netherlands), which resulted in much less microporosity. (IsoTis, the Netherlands). Granules of 30-50µm were sieved of both materials and two granules of each type were combined to form a treatment unit. These were further processed as one unit for seeding, implantation, explantation and histology. One week before implantation, four study groups were obtained by dynamically seeding 1,6E6 cells/unit overnight and subsequent static culturing for six days under different conditions: US- US+ AS+ USS- SF- Treated as US+ without cells.

Control Treated as US+ without cells.

Two additional groups were created 14-16 hours before implantation. The seeding density was increased 5.5 fold, to achieve cell numbers, as measured by bioreduction of tetrazolium salt (MTT, Sigma), equal to the 6-days cultured constructs:

US- Seeding medium identical to US-.
SF- Seeded in serum free medium on fibronectin coated scaffolds.

Units of all conditions were implanted separately in the paraspinal muscles of the goats the cells were derived from (n=8). After 12 weeks, the animals were killed and the samples were processed for undecalcified histology. Sections (10µm) were made with a sawing microtome and stained with basic fuchsin/methylene blue. On a section through the implant center, the percentage of bone area in the pores was measured using a VIDAS system coupled to a light microscope. Statistical analysis was done by a Friedman paired rank test and post-hoc Wilcoxon signed rank test (significance was set at p=0.05).

Results

Bone was found in amounts varying between the goats in all treatment groups except for the controls. The histomorphometric results are shown in figure 1. In the 35 units where bone had formed (87% of all 40 TE constructs) the HA70/800 yielded more bone than the HA60/400 in 33 cases (p<0.01). Statistical analysis demonstrated that the culture/seeding condition significantly influenced the bone area percentage, also when considering only the HA70/800 granules. To analyze the effect of ECM after 6 days culturing without osteogenic stimulation, post-hoc analysis of the US- condition (9% bone) versus the USS- condition (3% bone) indicated a significant difference (p=0.028). The effect of osteogenic stimulation, serum type, or serum free seeding were not tested, as these appeared minimal as can be seen in the histogram

Figure 1: Percentage of bone in the pores of the different scaffold types after 12 weeks i.m. implantation of TE constructs in the goat. (Error bars indicate the standard deviation)

Discussion / conclusion

The most important observation of this study is that all tissue engineering strategies we used can generate bone ectopically in the goat. In the same goats the HA material without cells failed to show bone formation making an osteoinductive mechanism unlikely. In the present study, the type of scaffold appeared to affect the outcome more than any of the TE parameters. The most prominent difference between the scaffolds was the higher microporosity in the HA70/800, that might have resulted in increased dissolution and precipitation with the possible integration of inducing factors. Unexpectedly, neither the serum type nor osteogenic stimulation had a large effect on bone formation. We did find an advantageous effect of culturing the constructs before implantation. Likely this was the result of the ECM that had formed on the scaffold as the cell numbers were normalized. In this study, different TE strategies as presented in different reports were deduced to different parameters and compared for the first time in a large animal model. The findings indicate an advantage for ECM and emphasize the need for an appropriate scaffold.

Acknowledgements

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References

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