THE OSTEOGENICITY OF AUTOLOGOUS CORTICAL BONE TRANSPLANTS

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Introduction
To engineer a successful alternative for autologous bone graft (AG), fundamental knowledge on its functioning is essential. Although considered as the golden standard, little is known about the specific functions that make the AG superior to the present alternatives. One such functions is the potential bone forming capacity of the osteoprogenitor cells within the AG (ostogenesis). However, survival of these cells is questionable, especially in grafts of clinically relevant size where vascularisation is absent during the first weeks after transplantation. It has been shown that cells can survive and function in relatively small implants placed subcutaneously in rats.1 To our knowledge however, this has never been shown in large mammals where survival can be expected to be more complicated. Even if cells survive, their function was not demonstrated in the few experiments that have compared vital and devitalized autografts in orthotopic transplants in large mammals.2,3 In these experiments however, vital and devitalized grafts were analyzed when they were fully incorporated by osteoconduction and not early after transplantation, when significant new bone formation as a function of surviving cells can be expected.

At present, much research is focusing on bone tissue engineering as an alternative for AG. The concept is to build a construct of osteoprogenitor cells with a biocompatible scaffold. In small animal models the potential of these cells was shown to be present in ectopically formed bone.4 Therefore, the success of tissue engineering is likely to rely on survival and subsequent functioning of these cells. As for the AG, this is questionable when applied in larger mammals. As a first step, to obtain a better knowledge regarding cell survival and the importance of this in larger mammals, we performed a study on AG bone in the goat. Our main research question focused on the relationship between vitality of AG and both ectopic and orthotopic bone formation.

Materials and Methods
After approval of the animal care committee, eight 2-year old Dutch milk goats were operated on twice. At the first operation autologous cortical bone grafts were prepared from a 2.3cm cortico-non-periosteal right femoral segment, excised under constant cooling. The segment was sectioned in the sagittal and frontal plane to provide four grafts with a volume of approximately 0.9cm3 each. Per goat the grafts were treated to provide: (1) vital cortex – no specific treatment –; (2) devitalized cortex – by freezing twice in liquid nitrogen –; (3) morselized vital cortex – by mortar and pestle (2x3mm chips) – and (4) morselized devitalized cortex. The efficiency of devitalization was analyzed by culturing eight devitalized morselized grafts for 30 days to detect for adherent cells and by a 48 hour alamarBlue® (Biosource, US) assay to detect metabolic activity. After preparation the grafts were randomly allocated to one of four paraspinous intramuscular pockets. Fluorochrome labels (Sigma, the Netherlands) were administered intravenously after 5 weeks (Alizarin Red), 7 weeks (Calcine green), and 9 weeks (Xylenol orange). Eight weeks after the first operation the goats were again operated on. Corticocancellous plugs (∅9.5x10mm) were taken from the medial and lateral condyle of the left femur with a hand trephine. One plug was devitalized, then both plugs were morselized and implanted in the opposing location. Explantation and implantation operative order and origin of the devitalized plug were randomized. The animals were killed 4 weeks later. The explants were processed for non decalcified histology and stained with basic fuchsin/methylene blue to allow discrimination of old dead bone (pale empty lacunae) from new bone (bright red, cellular). Unstained sections were used for fluoroscopy. Histomorphometry on percentage new bone formation on central sections by a VIDAS image analysis system coupled to a light microscope. Differences were analyzed by two tailed, paired student t-tests.

Results
Devitalisation was efficient as no adherent cells were detected and no metabolic activity could be measured. At explantation of the ectopic implants all devitalized morselized grafts had been resorbed. All other implants could be located within the muscle without signs of inflammation. The vital morselized cortical implants typically had formed compact ossicles. The devitalized intact cortical implants had decreased in size substantially as compared to the vital intact cortical implants. Histology showed new bone formation in all retrieved implants ranging from minute spots on the devitalized cortex to extensive bone formation bridging between individual chips of the vital morselized grafts. Flurochrome analysis of the vital morselized grafts indicated the occurrence of mineralisation at 5, 7 and 9 weeks. Within the vital intact cortical implants most bone was laid down on the walls of resorbed harversian channels. Only the 7 and 9 week labels were found. For devitalized intact cortex, little bone formed on the bottom of peripheral resorption pits with only the 9 week label showing. Histomorphometry indicated 10.3±5% (Mean area% ±SD) newly formed bone in the vital cortical explants, compared to 1.7±1.2% in the devitalized cortex (p<0.01 see Fig.1a). The one-month orthotopic implants (vital and devitalized) appeared well integrated in the defect. In both conditions the grafted cancellous bone was partially covered with new bone. Histomorphometry indicated more new bone inside the defect when the grafts were vital (p=0.02 Fig 1b). The label given one week after implantation was occasionally present in both conditions.

Discussion/conclusions
In the present study we clearly demonstrated the effect of AG vitality on both ectopic and orthotopic bone formation. We devitalized control grafts before implantation by freezing in liquid nitrogen. Contrary to other devitalizing methods no negative effect on osteoinductive capacity has been reported for this method. In the intact cortical ectopic grafts, the amount of new bone was significantly higher in the vital implants. Furthermore, mineralisation identified by fluorochromes was 4 weeks earlier when compared to the devitalized grafts, suggesting an early osteogenic mechanism. These findings agree with a study in which immunohistochemistry identified human cells in new bone formed on human bone grafts in mice.5 Unfortunately this formal proof of osteogenesis was not possible in our model. The influence of vitality was more pronounced with cortical bone than with orthotopic cancellous bone. Osteoconduction in the orthotopic location may account for this difference. In conclusion the findings indicate that a proportion of the cells inside the autografts survived long enough to promote new bone formation, most probably by osteogenesis. With respect to tissue engineering of bone this is a promising observation.

Acknowledgements
The authors acknowledge The Netherlands Technology Foundation (STW; grant UGN.4966) for financial support.

Literature

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