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ORIGINAL ARTICLE

Assessment of activated porous granules on implant fixation and early bone formation in sheep

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Summary *Background/Objective:* Despite recent progress in regeneration medicine, the repair of large bone defects due to trauma, inflammation and tumor surgery remains a major clinical challenge. This study was designed to produce large amounts of viable bone graft materials in a novel perfusion bioreactor to promote bone formation.
Methods: Cylindrical defects were created bilaterally in the distal femurs of sheep, and titanium implants were inserted. The concentric gap around the implants was randomly filled either with allograft, granules, granules with bone marrow aspirate (BMA) or bioreactor activated granule (BAG). The viable BAG consisted of autologous bone marrow stromal cells (BMSCs) seeded upon porous scaffold granules incubated in a 3D perfusion bioreactor for 2 weeks prior to surgery. 6 weeks after, the bone formation and early implant fixation were assessed by means of micro-CT, histomorphometry, and mechanical test.
Results: Microarchitectural analysis revealed that bone volume fraction and trabecular thickness in the allograft were not statistically different than those (combination of new bone and residue of granule) in the other 3 groups. The structure of the allograft group was typically plate-like, while the other 3 groups were combination of plate and rod. Histomorphometry showed that allograft induced significantly more bone and less fibrous tissue in the concentric gap than the other 3 granule groups, while the bone ingrowth to implant porous surface was

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not different. No significant differences among the groups were found regarding early implant mechanical fixation.
Conclusion: In conclusion, despite nice bone formation and implant fixation in all groups, bioreactor activated graft material did not convincingly induce early implant fixation similar to allograft, and neither bioreactor nor by adding BMA credited additional benefit for bone formation in this model.

Introduction

The early bone ingrowth to porous surface of implants increases primary implant fixation and reduces the risk of implant failure [1]. Osseointegration is influenced by the primary mechanical stability and secondary biological stability after bone remodelling of the implant in the bone. Thereby, early bone formation and apposition is essential for secondary stability [2].

Despite recent progress in regeneration medicine, the repair of large bone defects due to trauma, inflammation, and tumour surgery remains a major clinical challenge. Large animal models have been developed to test bone repair by tissue engineering approaches that combines the principles of engineering and life sciences to overcome drawbacks of traditional bone regeneration techniques used in orthopaedics [3]. These new techniques are intended to develop the tissue-engineered constructs with similar structural and mechanical characteristics of natural bone [4]. In general, an advantage in the bone regeneration in large defect has often been reported when scaffolds were seeded with bone marrow derived stromal cells (BMSCs) [5].

Consequently, bioreactor is introduced. This device enables a closely monitored and tightly controlled environment to allow biological and biochemical processes. Bone tissue engineering consists of static cultures, in which bone cells are seeded on a 3-dimensional (3D) scaffold and placed in a well-plate for a certain period of time. This culture strategy leads to significant drawbacks that the cells tended to accumulate at the periphery of the scaffold leading to poor nutrient and waste exchange in the centre of the scaffold [6]. Furthermore, cell necrosis could also be formed in the centre of the scaffold. Dynamic bioreactors such as perfusion bioreactors might overcome such problems in cell culture.

A perfusion bioreactor is developed and designed to mimic the microscopic mechanical loading of bone *in vivo* [7]. Perfusion bioreactor systems automatically pump culture medium through the interconnected pores of scaffold that is press-fitted into a culture chamber [8—10]. With flow perfusion, the mass transfer is enhanced at the interior of the 3D scaffold and shear forces are applied to the cultured cells. Compared with other bioreactor systems, a perfusion bioreactor increases mass transport leading to improved distribution of extracellular matrix throughout the 3D scaffold, increased cell number, enhanced expression of the osteogenic phenotype, and improved mineralized extracellular matrix deposition [9,11,12]. It is worth noting that only the perfusion bioreactor is able to eliminate diffusion limitations inside a scaffold, although dynamic bioreactors can overcome diffusion limitations at the surface of a scaffold [9,13]. Hence, the perfusion bioreactor seems to be a very useful dynamic culture technique for bone tissue engineering [4,6], and is the current most commonly used dynamic bioreactor [14].

This study was designed to produce viable bone graft material for early fixation of titanium alloy implants to bone defects. The procedures described and used aimed at mimicking the possible future clinical setting, where autologous BMSCs were harvested and implanted with a biomaterial around a critical defect created in association with a revision of a total joint arthroplasty (fluidised bed concept). We hypothesised that filling with the bioreactor activated graft material (BAG) in a 2 mm defect in sheep bone would result in ingrowth and bone formation comparable to allograft.

Materials and methods

Study design

This study used a well-validated bilateral implant-gap defect model that has been described in detail previously [15,16]. Eight sheep were included based on sample size calculation and our previous experience. Cylindrical defects were created laterally and medially in the bilateral distal femoral condyles of each sheep. This allowed bilateral insertion (press fit) of titanium alloy implants extra-articularly in the distal femurs; thus, four implants per sheep. The concentric defect around the inserted titanium alloy implant was filled with one of the four materials: (1) allograft serving as control; (2) granule; (3) granules incubated with fresh autologous bone marrow aspirate (BMA); or (4) granules incubated for 2 weeks in a bioreactor with autologous BMSCs. The filling materials were alternated between insertion holes in order to avoid any site dependent differences. This randomisation allowed all four materials to be implanted medially or laterally within the same sheep.

Animals and bone marrow aspiration

This study was approved by the Danish Animal Experiments and Inspectorates (no. 2008/561-1544), and all animal