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Autologous vs. allogenic mesenchymal progenitor cells for the reconstruction of critical sized segmental tibial bone defects in aged sheep

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ABSTRACT

Mesenchymal progenitor cells (MPCs) represent an attractive cell population for bone tissue engineering. Their special immunological characteristics suggest that MPCs may be used in allogenic applications. The objective of this study was to compare the regenerative potential of autologous vs. allogenic MPCs in an ovine critical size segmental defect model. Ovine MPCs were isolated from bone marrow aspirates, expanded and cultured with osteogenic medium for 2 weeks before implantation. Autologous and allogenic transplantation was performed using the cell-seeded scaffolds and unloaded scaffolds, while the application of autologous bone grafts served as a control group ($n = 6$). Bone healing was assessed 12 weeks after surgery by radiology, microcomputed tomography, biomechanical testing and histology. Radiology, biomechanical testing and histology revealed no significant differences in bone formation between the autologous and allogenic groups. Both cell groups showed more bone formation than the scaffold alone, whereas the biomechanical data showed no significant differences between the cell groups and the unloaded scaffolds. The results of the study suggest that scaffold-based bone tissue engineering using allogenic cells offers the potential for an off-the-shelf product. Thus the results of this study serve as an important baseline for translation of the assessed concepts into clinical applications.

1. Introduction

The most suitable cell source for scaffold-based bone engineering is still the focus of much debate in the literature. There is no denying the potential of including a cell population within a tissue engineered construct (TEC) which is able to regenerate the host site, however, the best approach from a clinical point of view is yet to be determined.

Several cell-based strategies aim to improve osteoinduction by the incorporation of cells with a high osteogenic differentiation potential, such as bone marrow derived mesenchymal progenitor cells (MPCs). Gronthos et al. [1] have defined these cells as multipotent progenitor cells which have the potential to differentiate into a variety of mesenchymal tissues such as bone, cartilage, tendon, ligaments, muscle, fat and dermis [2–5]. MPCs can be isolated from a variety of tissues [6–8] using different separation techniques and can be differentiated into the appropriate phenotype under defined culture conditions and the action of specific growth factors or cytokines [9]. These cells have shown their therapeutic potential in a number of in vivo studies for the regeneration of large bone defects and non-unions [10–13]. The supply of autologous MPCs is often limited and the pre-operative preparations for effective isolation, expansion and differentiation is time consuming and labour intensive. For this reason, to acquire an adequate number of cells for transplantation the time period between cell isolation and cell transplantation is usually at least 4–6 weeks. Consequently, the major drawback of using an autologous cell source is two pronged; limitations on cell numbers when utilizing them immediately after extraction or the long time period and associated costs which are necessary to expand the cells in vitro until a suitable number is attained. However, the special immunological characteristics of MPCs suggest that they could in fact be used successfully for non-autologous applications in bone tissue engineering [14,15]. Allogenic cell transplantation is a common therapeutical option and is in routine clinical use in the field of oncology [16,17]. Translating the idea of allogenic cell transplantation from oncology to orthopaedics could offer a new opportunity for the use of MPCs for regenerative medicine as an "off-the-

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shelf product". Before translating these new treatment concepts into clinical applications in orthopaedic and trauma surgery rigorous evaluation of the respective cell populations in an appropriate preclinical animal model are essential [18,19].

Several animal models have been developed over the years to verify the practicality of different research bone regeneration concepts [20–22]. Among these adult sheep offer the advantage of having a comparable body weight, a similar mineral composition of bone and similar metabolic and remodelling rates to humans and, furthermore, long bone dimensions which allow the use of human implants and prostheses, which is not possible in smaller species [23,24]. Thus our group has recently established a challenging ovine segmental bone defect model using 6- to 7-year-old animals, which at this age display secondary osteon remodelling, characteristic of human bone. In our recent more clinically driven strategies we have moved towards defining an appropriate cell source for bone tissue engineering to circumvent the aforementioned disadvantages associated with autologous cell transplantation in favour of allogenic MPC sources [25].

We hypothesize that allogenic MPCs do not show a clinically detectable immune response and have similar osteogenic potential to autologous MPCs in scaffold–cell based bone engineering. Thus the aim of the current study was to assess and compare the regenerative potential of autologous versus allogenic MPCs in combination with a mPCL–TCP scaffold in a critical sized segmental bone defect in sheep.

2. Materials and methods

All reagents and consumables were purchased from Sigma–Aldrich unless stated otherwise.

2.1. Scaffold fabrication and preparation

Biodegradable scaffolds comprising medical grade polycaprolactone (80 wt.%) and β-tricalcium phosphate (20 wt.%), (mPCL–TCP) (outer diameter 20 mm, height 30 mm, inner diameter 8 mm) (Fig. 1F) were fabricated by fused deposition modelling (FDM) (Osteopore International, Singapore) (for design details see Supplementary material). Prior to surgery all scaffolds were surface treated for 6 h with 1 M NaOH and washed five times with phosphate-buffered saline (PBS) to render the scaffold surface more hydrophilic. Scaffold sterilization was achieved by incubation in 70% ethanol for 5 min followed by complete evaporation and subsequent UV irradiation for 30 min.

2.2. Biomechanical testing of scaffold and internal fixation

To investigate the mechanical behaviour of the implant–bone–scaffold construct biomechanical testing was performed in vitro on six specimens (for details see Supplementary material).

2.3. Cell harvesting

Ovine MPCs were obtained from 6- to 7-year-old Merino sheep undergoing experimental surgery. Bone marrow aspirates were obtained from the iliac crest under general anaesthesia (Fig. 1A). Total bone marrow cells (5–15 $\times$ 10^6 cells ml^{-1}) were plated at a density of 10–20 $\times$ 10^6 cells cm^{-2} in complete medium consisting of low glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Cells were subsequently plated at a density of 10^3 cells cm^{-2} (Fig. 1B). We have previously demonstrated that MPCs express the respective phenotypic profiles typical of different mesenchymal cell populations and show a multilineage differentiation potential [26]. 2 weeks before implantation the medium was changed to osteogenic medium (DMEM, 10% FBS, 100 U ml penicillin and 100 μg ml streptomycin, 10 μl ml β-glycerophosphate, 1 μl ml ascorbic acid and 1 μl ml dexamethasone) to induce osteogenic differentiation (Fig. 1C). For three-dimensional (3-D) cultures, 35 $\times$ 10^6 ovine MPCs suspended in 500 μl of basal

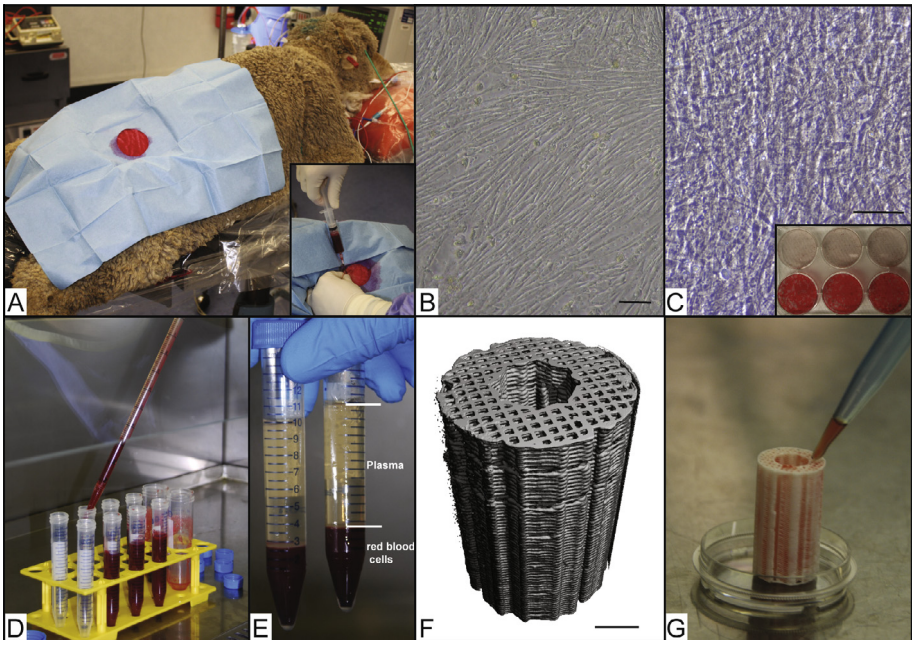

Fig. 1. (A) Bone marrow aspiration (10 ml) from the iliac crest was performed under general anaesthesia. (B) MPCs were typically elongated in shape after culture for 10–14 days in expansion medium (scale bar 100 μm). (C) The cell shape changed to a more compact cobblestone-like appearance within days after being exposed to osteogenic media (scale bar 100 μm). (Insert) Alizarin red staining of MPC cultures after 14 days in 6-well plates. Under osteogenic conditions MPCs secreted a mineralized matrix, whereas the control cultures did not reveal any staining. (D) To prepare PRP blood was collected from the jugular vein of the sheep, mixed, and transferred into falcon tubes. (E) After centrifugation at 2400 rpm for 20 min the plasma was removed and centrifuged a second time. (G) The resulting pellet was resuspended in 1.2 ml of plasma, and the cells in combination with PRP were seeded onto the scaffolds. (F) A micro-CT image of the cylindrical mPCL–TCP scaffold produced via fused deposition modelling for segmental bone defect repair (scale bar 5 mm).