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A tissue engineering strategy for the treatment of avascular necrosis of the femoral head

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ABSTRACT

Background & purpose: Skeletal stem cells (SSCs) and impaction bone grafting (IBG) can be combined to produce a mechanically stable living bone composite. This novel strategy has been translated to the treatment of avascular necrosis of the femoral head. Surgical technique, clinical follow-up and retrieval analysis data of this translational case series is presented.

Methods: SSCs and milled allograft were impacted into necrotic bone in five femoral heads of four patients. Cell viability was confirmed by parallel *in vitro* culture of the cell-graft constructs. Patient follow-up was by serial clinical and radiological examination. Tissue engineered bone was retrieved from two retrieved femoral heads and was analysed by histology, microcomputed tomography (μCT) and mechanical testing.

Results: Three patients remain asymptomatic at 22- to 44-month follow-up. One patient (both hips) required total hip replacement due to widespread residual necrosis. Retrieved tissue engineered bone demonstrated a mature trabecular micro-architecture histologically and on μCT. Bone density and axial compression strength were comparable to trabecular bone.

Conclusions: Clinical follow-up shows this to be an effective new treatment for focal early stage avascular necrosis of the femoral head. Unique retrieval analysis of clinically translated tissue engineered bone has demonstrated regeneration of tissue that is both structurally and functionally analogous to normal trabecular bone.

Introduction

AVN of the femoral head is a disease that usually affects young adults, progressing to bone collapse and osteoarthritis in over 80% of untreated patients.[1] Progression occurs even in 59% of asymptomatic patients.[2] Most cases are idiopathic but it is commonly attributed to steroid therapy, chemotherapy, alcohol or sickle cell disease, whilst the chance of developing AVN after traumatic hip dislocation may be as high as 40%.[3] Position and distribution of the necrotic bone in the femoral head have a bearing on prognosis[2,4] however the most important predictor remains progression from an intact bony architecture (Ficat & Arlet Stage I or II) to loss of normal bone structure and involvement of articular cartilage (Ficat & Arlet Stage III or IV).[5]

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Untreated cases therefore, even in the early and asymptomatic stages, have a high probability of requiring a total hip replacement (THR). Joint preserving therapies are therefore advocated by most authorities to prevent the progression to collapse. These include core decompression,[1] electrical stimulation,[6] tantalum trabecular metal rods,[7] vascularised fibular grafts,[8] fibular or tibial strut grafts,[9] concentrated autologous bone marrow[10] or hydroxyapatite rods coated with skeletal stem cells.[11]

Skeletal stem cells (SSCs) have been used for the treatment of avascular necrosis of the femoral head,[12] though this treatment does not provide any structural support for the overlying cartilage. Impaction bone grafting (IBG) can provide mechanical support and has been used as a void-filler in revision hip surgery for over forty years[13] and in the treatment of AVN.[14] Whilst some remodelling has been demonstrated on histological specimens from IBG in acetabula and femora, areas of non-incorporated graft with associated necrosis, fibrocartilage and fibrosis remain in all specimens.[15–17]

SSCs can be combined with IBG to improve both the mechanical and biological characteristics of the graft.[18] This novel technique has been translated from the laboratory to the clinic for the treatment of early stage AVN. We report our case series from this novel tissue engineering strategy. Two femoral heads, both in the same patient, have collapsed requiring THR. This has however provided the opportunity for retrieval of the human tissue engineered bone and for unique *ex vivo* analysis.

Materials and methods

Surgical technique

Milled allograft was prepared from fresh frozen femoral heads according to standard clinical practice. The patient was positioned laterally on the operating table and bone marrow was aspirated from the posterior iliac crest, rotating and re-angling the needle regularly to minimise contamination with venous blood. The marrow was concentrated in theatre by calibrated centrifugation (Marrowstim, Biomet, Swindon, UK) to isolate the nucleated cell fraction. This provided a concentrated pool of pleuripotent SSCs,[18] which was mixed with the prepared allograft. The patient was then placed supine on the operating table to allow fluoroscopic guidance of instrumentation up the femoral neck into the necrotic area of the femoral head. Through a 2 cm incision in the lateral thigh, a channel was drilled over a guidewire into the sub-chondral bone and the necrotic bone was removed by curettage. Cell-seeded milled allograft was impacted retrograde into the channel using a 12 mm diameter Xchange tube saw (Stryker, Newbury, UK). Samples of allograft/SSC mix were retained for parallel *in vitro* analysis of cell viability. Patients maintained protected weight bearing for 6 weeks post-operatively and follow-up was by serial radiological and clinical examination.

Patient cohort

Four patients, all with bilateral AVN, were treated at our institution using this tissue engineering strategy. Three of the patients presented with advanced disease in one hip, requiring contra-lateral THR (Table 1).

Parallel in vitro *assessment*

The retained samples were cultured in basal media for 14, 28 and 42 days, with twice weekly media changes, prior to staining with CellTracker Green and ethidium homodimer (CTG-EH). Microscope images were recorded using Carl Zeiss Axiovision software Ver 3.0 via an AxioCam HR digital camera on an Axiovert 200 inverted microscope (Carl Zeiss Ltd, Welwyn Garden City, UK) under fluorescent light.

Retrieval of specimens

In Patient 4, both femoral heads progressed to collapse requiring bilateral THR, on the right after 13 months and on the left after 19 months. The femoral heads were retrieved, with patient consent and prior ethical approval (LREC194/99/1), photographed and fixed in 4% paraformaldehyde (PFA) prior to further analysis.

Microcomputed tomography (μCT)

The retrieved femoral heads were scanned using an Xtek Benchtop 160Xi scanner (Xtek Systems Ltd, Tring, UK) equipped with a Hamamatsu C7943 X-ray flat panel sensor (Hamamatsu Photonics, Welwyn Garden City, UK). Scan resolution was up to 31-μm, at 150 kV, 60 μA using a molybdenum target with an exposure time of 534 ms and 4× digital gain. Reconstructed volume images were analysed using VGStudio

Table 1 – Patient details.

Patient	Age	Attributed cause	Right hip stage[a]	Right hip treatment	Left hip stage[a]	Left hip treatment
1	42	Systemic steroids for sub-arachnoid haemorrhage	IV	THR	II	SSC/IBG
2	40	Alcohol	III	THR	II	SSC/IBG
3	31	Idiopathic	III	THR	II	SSC/IBG
4	32	Systemic steroids for testicular carcinoma	II	SSC/IBG	II	SSC/IBG

[a] = Ficat & Arlet Classification is used throughout this study unless otherwise stated.