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Chondrogenically primed mesenchymal stem cell-seeded alginate hydrogels promote early bone formation in critically-sized defects

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ABSTRACT

Hypertrophic cartilaginous grafts can be engineered *in vitro* using bone marrow derived Mesenchymal Stem Cells (MSCs). When such engineered tissues are implanted *in vivo* they have been shown to induce bone formation by recapitulating aspects of the developmental process of endochondral ossification. Alginate, a naturally sourced and biocompatible hydrogel, offers an attractive 3D environment to facilitate the *in vitro* chondrogenesis of MSCs. Furthermore, such alginate hydrogels can potentially be used to engineer cartilage tissues of scale to promote endochondral bone regeneration in large bone defects. The aim of this study was to investigate the ability of chondrogenically-primed MSC-laden alginate hydrogels to induce healing in two distinct critically-sized defect models. Bone marrow derived MSCs were seeded into alginate hydrogels, chondrogenically primed *in vitro* in the presence of TGF-β3 and then implanted into either a critically-sized rat cranial or femoral defect. μCT analysis 4 weeks post-implantation revealed significantly higher levels of mineralization within the femoral defects treated with MSC-laden alginate hydrogels compared to untreated empty controls, with similar results observed within the cranial defects. However, any newly deposited bone was generated appositional to the alginate material, and occurred only superficially or where the alginate was seen to degrade. Alginate material was found to persist within both orthotopic locations 8 weeks post-implantation, with its slow rate of degradation appearing to prevent complete bone regeneration. In conclusion, while chondrogenically primed MSC–alginate constructs can act as templates to treat critically-sized defects within bones formed through either intramembranous or endochondral ossification, further optimization of the degradation kinetics of the hydrogel itself will be required to accelerate bone tissue deposition and facilitate complete regeneration of such defects.

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1. Introduction

Critically-sized bone defects, beyond the self-regenerating ability of the body, are commonly occurring clinical problems which may arise from issues such as trauma, bone disease and cancer. The repair of such defects typically relies on the use of bone grafting, whereby bone can be sourced from within the patient's own body (an autograft), or from donated bone (an allograft). Both approaches have associated limitations, including the lack of available autograft bone, the additional risk, pain and trauma due to harvesting this bone, and the risk of an immune rejection of donated tissue. Tissue engineering or regenerative medicine aims to provide an alternative source of grafting material, by engineering a suitable tissue *via* the combination of scaffolding materials, cells and bioactive signals *in vitro* which can then be implanted *in vivo* to support or enhance the self-healing process of the body [1,2].

Initial attempts to engineer such tissues focused on the direct generation of a bone-like material by inducing osteogenesis of Mesenchymal Stem Cells (MSCs) or other osteoprogenitor cells; a strategy resembling the developmental process of intramembranous ossification. However some problems have arisen following implantation of such tissues, including a lack of vasculature in-growth, core degradation and necrosis due to a lack of available nutrients. This may be attributed to the establishment of a densely mineralized matrix within these constructs prior to implantation which may act to inhibit the ingrowth of host vessels [3,4]. More recently, efforts have turned to engineering a cartilage-like tissue *in vitro* in an attempt to recapitulate the process of endochondral ossification, whereby a cartilage template is converted to mature bone. This is the developmental process through which long bones form, and is the typical healing response observed during long bone fracture repair [5,6]. During endochondral ossification, chondrocytes in a cartilage rudiment undergo hypertrophy and begin to secrete signals which induce the ingrowth of a vasculature network and the mineralization of the surrounding matrix. Therefore, the implantation of tissue engineered cartilage is conceived as a novel strategy to promote endochondral ossification within critically-sized defects, eventually leading to the establishment of a vascularized, mineralized and mechanically functional extracellular matrix [7].

The potential of this approach has been demonstrated using MSC-derived engineered cartilaginous grafts in both ectopic (subcutaneous) environments and within orthotopic defects [8–14]. Challenging orthotopic defects often require treatment with a construct of specific and large dimensions, which will have to be engineered and cultured *in vitro* prior to implantation [15]. The use of a supporting scaffold or hydrogel can facilitate the scaling-up of such engineered constructs to clinically relevant sizes [16–22], and the choice of a suitable 3D material is vital to ensure success. Appropriate biomaterials should not elicit a rejectory immune response, should provide sufficient mechanical support to allow for surgical implantation, should induce a favourable reaction from implanted or host cells, and it should degrade at a suitable rate to be replaced by newly forming tissue. Therefore identification of a suitable scaffolding material to tissue engineer scaled-up cartilage will be central to the successful realization of endochondral bone tissue engineering strategies.

Alginate is a naturally derived hydrogel material which offers many advantages for use in such tissue engineering applications including excellent biocompatibility and ease of gelation to form constructs with specific dimensions [23–25]. Robust chondrogenesis of MSCs has been achieved using alginate hydrogels [26–28], and it has also been shown to act as a suitable template for ectopic bone growth [29,30]. This hydrogel has also been successfully used as a growth factor delivery system to facilitate regeneration of bone defects [31,32].

We have recently demonstrated that cartilaginous tissues engineered using MSC-laden alginate hydrogels promote ectopic bone formation following subcutaneous implantation into nude mice [30,33]; however the capacity of such constructs to accelerate the regeneration of critically-sized orthotopic bone defects has yet to be assessed. The objective of this proof of concept study was therefore to determine the efficacy of MSC-laden alginate gels to undergo chondrogenesis *in vitro* and induce osteogenesis within critically-sized cranial and femoral bone defects *in vivo*. These two models were selected to identify any differential response of healing within bones which formed through different pathways during skeletogenesis, with the cranium developing through the intramembranous pathway and the femur forming by endochondral ossification. To facilitate vascularization and mineralization of these constructs *in vivo*, the architecture of the alginate hydrogels was also modified to include channels which have previously been shown to accelerate mineralization of engineered cartilage grafts in ectopic locations [34]. *De novo* bone formation was investigated using microCT to identify and quantify mineralization, and histological analysis was used to evaluate the nature of the repair tissue within untreated and treated defects.

2. Materials and methods

2.1. Isolation and expansion of MSCs

Bone marrow derived MSCs were isolated from the femoral shaft of Fischer rats and expanded in expansion medium (high glucose Dulbecco's modified eagle's medium GlutaMAX (hgDMEM) supplemented with 10% v/v foetal bovine serum (FBS), 100 U/mL penicillin – 100 μg/mL streptomycin (all Gibco, Biosciences, Dublin, Ireland), 2.5 μg/mL amphotericin B (Sigma–Aldrich, Dublin, Ireland) and 5 ng/mL human fibroblastic growth factor-2 (FGF-2; Prospec-Tany TechnoGene Ltd., Israel) and expanded to passage 2 at 20% pO_2.