

BIOREACTOR-BASED ENGINEERING OF OSTEOINDUCTIVE GRAFTS

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INTRODUCTION

A major challenge to be faced in order to introduce cell-based therapies for bone repair into wide-spread surgical practice is to translate a research-scale production model into a manufacturing design that is reproducible, clinically effective, and economically viable. One possible means by which to achieve this goal is via a bioreactor system capable of controlling, automating, and streamlining all of the individual phases of the bone-tissue engineering process.

Bone marrow stromal cells (BMSC) can easily be expanded in monolayers from a marrow aspirate, and when loaded into porous ceramic scaffolds, are capable of generating osteoinductive constructs [1], which have been demonstrated to support bridging of large segmental bone defects in humans [2]. Moreover, when BMSC are cultured under controlled regimes of perfusion, after monolayer expansion, the cells have been shown to have an increased capacity to differentiate and deposit mineralized matrix [3].

We have previously described a bioreactor for efficient and uniform cell seeding of 3D scaffolds under direct oscillating perfusion, and demonstrated its applicability to seed expanded BMSC on ceramic scaffolds [4]. Working towards the ultimate goal of automating and streamlining the process of generating osteoinductive grafts, in this work we combined both the seeding and culturing of BMSC into porous ceramic scaffolds within a single bioreactor, and eliminated the phase of monolayer expansion. Specifically, we used this bioreactor to test whether under perfusion i) BMSC could be seeded directly into a porous ceramic scaffold, starting from nucleated cells freshly isolated from a bone marrow aspirate; ii) BMSC would proliferate within the ceramic; and iii) the resulting constructs would be osteoinductive upon in vivo grafting.

METHODS

Bone marrow aspirates were obtained from four adult donors (36 to 54 years old) during routine orthopaedic surgical procedures involving

exposure of the iliac crest, in accordance with the local ethical committee and after informed consent. Nucleated cells were separated by Ficoll density gradient centrifugation and resuspended in medium supplemented with 10% FBS, 10^{-8} M Dexamethesone, 5ng/ml FGF-2, and 10^{-4} M ascorbic acid. Porous hydroxyapatite ceramic scaffolds (Finceramica, Faenza, Italy) were 8mm diameter by 4mm thick disks. An average of 18 million nucleated cells per scaffold were added to the bioreactor, and the cell suspension perfused through the pores of the ceramics at a superficial velocity of 1mm/s. After 5 days, medium was replaced, perfusion flow was reduced to 0.25mm/s, and constructs were cultured for up to an additional 2 weeks in the bioreactor. Following in vitro culture, one half of each ceramic was used to extract cells, count them and assess their phenotype by cytofluorimetry and clonogenicity by colony forming efficiency tests. The other half of each ceramic was implanted subcutaneously in a nude mouse for 8 weeks and subsequently histologically assessed by hematoxylin and eosin stain.

RESULTS

Colony forming efficiency assays indicated that the initial concentration of BMSC in the nucleated cell fraction of the marrow aspirates averaged 0.026%. The numbers of BMSC seeded within the ceramics thus averaged 4800 and increased over the culture period, up to an average of $9.0E+05$ BMSC per ceramic after 19 days of perfusion. Based on the determined number of clonogenic BMSC initially loaded, this corresponded to an average expansion of 8.2 doublings. Cytofluorimetry analyses indicated the presence in the constructs of an additional component of hematopoietic cells (average of $4.2E+05$ per ceramic), which could have played a role in conditioning the expansion of BMSC. BMSC extracted from the ceramic constructs were highly clonogenic (29% to 38%), in contrast to those typically expanded in monolayers (11%). When BMSC-ceramic constructs from each donor were

cultured under perfusion for 19 days, abundant bone tissue was observed throughout the ceramics, both in the peripheral and central regions.

DISCUSSION

In this study, we have demonstrated for the first time that BMSC can be seeded by perfusion onto porous ceramic scaffolds directly from the nucleated cell fraction of a bone marrow aspirate, without a prior monolayer expansion phase. The seeded BMSC proliferated within the ceramic during the in vitro culture period and retained a higher clonogenicity than monolayer expanded BMSC, possibly suggesting that they remained in a more 'progenitor-like' state. The reproducible, abundant and uniform bone formation by

engineered constructs establishes that osteoinductive tissues for possible clinical use could be generated using autologous cells in a streamlined manufacture process based on perfusion systems [5]. Including monitoring and controlling features in the perfusion bioreactor will represent the next step to potentially provide an economically viable approach to the automated production of osteoinductive grafts [6].

REFERENCES

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