INFLUENCE OF CASSETTE DESIGN ON THREE DIMENSIONAL PERFUSION CULTURE OF ARTIFICIAL BONE IN VITRO

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Introduction

By nutrient transport and mechanical stimulation, perfusion culture systems have been proven to be effective bioreactors for in vitro construction of tissue-engineered bone. In current systems, cassettes for holding the scaffolds were usually fit tightly around the edge of scaffolds, so that the media flow was ensured to perfuse through scaffold internally instead of around the scaffolds. However, this press-fit method might also deplete the external flow around side surface of the scaffold, so as to result in an unviable cassette-scaffold interface. The cassettes might play a role as important as perfusion bioreactor itself to determine the chemotransport profile for a cultured construct. In this study, cassettes with internal ring-bar structures were proposed to improve external flow below and vitalize the interface of the artificial bone by perfusion culture for better regenerative potentials.

Materials and Methods

Mouse osteoblast-like MC 3T3-E1 cells were seeded at 1.5×10^6 cells/100 μ l and cultured in porous ceramic beta-tricalcium phosphate scaffolds (10 mm in diameter, 8 mm in height) for 3 days by either static culture or perfusional methods in an oscillatory perfusion bioreactor with 3 kinds of cassettes: conventional press-fitting cassettes, cassettes with 1 internal ring-bar, and cassettes with 2 internal ring-bars (Figure1). The cell proliferation, distribution and viability and early osteogenesis were evaluated. Differences among experimental groups were assessed by ANOVA residual analyses, and considered statically significant when $p < 0.05$.

Figure 1 Schematic of perfusion system and experimental grouping.

Results and Discussion

The results of calcein-AM/PI staining (Figure 2) showed that the bone grafts cultured by conventional perfusion method with press-fitting cassettes had poor cell viability on

the cassette-scaffold interface, which was even inferior to static culture; by contrast, the bone grafts cultured by modified cassettes with 1 or 2 internal ring-bars had a uniform distribution of living cells not only throughout the internal pores, but also on the whole external surface, which might be due to the improved external medium flow by the modification of internal structure of the cassettes.

Figure 2 Calcein-AM /PI double staining of midline section: A) Gloss view; B) with 10 $\times$ objective.

The total ALP activity in oscillatory perfusion culture was significantly higher than static culture ($p < 0.05$), but there was no significant difference among the three perfusional methods by different cassette designs.

Conclusions

Conventional perfusion culture method could not produce an ideal bone graft in vitro with good interface; whereas, by the new cassette design strategy, critical-sized tissue engineering bone could be cultured uniformly, with good cell viability both externally and internally, in the oscillatory perfusion culture system by only 1.5ml culture media

References

Dajiang DU, Katsuko FURUKAWA and Takashi USHIDA. Oscillatory Perfusion Seeding and Culturing of Osteoblast-like Cells on Porous Beta-Tricalcium Phosphate Scaffolds. The Journal of Biomedical Materials Research: Part A, 2007. (In press)