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Tissue engineered cartilage: Utilization of autologous serum and serum-free media for chondrocyte culture☆

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Summary

Background: Standard culture medium contains bovine serum. If standard culture methodology is used for future human tissue-engineering, unknown risks of infection from bovine disease or immune reaction to foreign proteins theoretically might occur. In this study we wished to evaluate the potential of chondrocyte expansion using autologous and serum free media.
Methods: Autologous auricular cartilage was harvested in a swine model. An initial concentration of 100×10^3 cells per group were expanded in three groups. Group A, F-12 with 10% fetal calf serum; Group B, F-12 supplemented with 10% autologous serum; Group C, F-12 supplemented with growth factors. Cell numbers were counted at days 3, 6, 9 and 12.
Results: The cells in all the three groups exhibited normal chondrocyte morphology. At early time points there was a statistically significant difference in the number of cells between Group A and the two other groups ($p < 0.05$). By day 12, both Groups A and C demonstrated greater cell number as compared to Group B ($p < 0.05$).

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Conclusion: The results suggest that both autologous serum as well as serum free media might be substituted for the expansion of the number of chondrocytes, thus avoiding the potential need for a bovine serum supplement.

1. Introduction

As attempts to grow a complete human sized and shaped auricle by tissue engineering techniques have evolved, newer refinements and challenges become apparent. Autologous chondrocytes isolated from normal or microtia cartilage will be utilized as the donor source for the generation of human tissue-engineered cartilage for neo-auricular constructs [1—5]. Only a limited supply of donor cartilage is available, yet the generation of a large number of new cells in a practical time limit will be required. Various methods have been successfully developed to increase the yield of cultured chondrocytes [6—9]. These methods utilize the addition of animal serum (FBS: fetal bovine serum) and/or growth factors to the culture media as nutrients.

Several hypothetical, albeit minimal, risks might exist with regard to the use of FBS. Animal (bovine) derived serum carries the remote possibility of transmission of prion or viral disease and zoonoses contamination. Potential immunologic reactions due to the formation of antibodies to fetal calf serum proteins are also another concern when the tissue-engineered cartilage is generated for human use. Patients can be sensitized with an IgE response against bovine serum albumin leading to anaphylactic reactions [10]. For example, studies have shown that cellular cardiomyoplasty using cells cultured with bovine serum has associated significant malignant ventricular arrhythmias; the use of autogenous serum for myoblast re-implantation eliminated arrhythmia morbidity and mortality. One putative mechanism could be due to the contact of human cells with fetal bovine serum resulting in fixation of animal proteins on the cell surface, representing an antigenic substrate for immunological and inflammatory adverse events [11]. Studies have also shown immune response in burn patients who received keratinocyte grafting cultured in bovine serum by the detection of antibodies against bovine serum proteins [12,13]. Persistence of bovine serum proteins in the skin graft with a potential risk of a delayed immune reaction and graft rejection is also possible [14]. Perhaps these potential complications could be minimized by efforts to reduce the use of FBS and other animal products or to replace bovine nutrients with either synthetic alternatives or autologous serum from the patient. In this study we wished to compare the rates of proliferation of chondrocytes in order to evaluate the potential of autologous serum and serum free media for chondrocyte expansion.

2. Materials and methods

2.1. Chondrocyte isolation

According to the approved IRB protocol for animal study, auricular cartilage was harvested from two pigs under general anesthesia. Perichondrium was removed under sterile conditions and the cartilage was fragmented into small pieces; washed in phosphate-buffered saline (PBS) solution containing 100 U/L of penicillin, 100-mg/L of streptomycin and 0.25-mg/L of amphoterecin B (Gibco, Grand Island, NY); and digested with 0.3% collagenase II (Worthington Biochemical Corp., Freehold, NJ, USA) for 8—12 h. The resulting cell suspension was passed through a sterile 250 μm filter (Spectra/Mesh 146-426; Spectrum Medical Industries, Inc., Laguna Hills, CA). The filtrate was centrifuged and the resulting cell pellet was washed twice with copious amount of Dulbecco's PBS. Cell number and viability was determined by cell count using a hemocytometer and tryphan blue dye. The chondrocyte suspensions demonstrating cell viability in excess of 85% were used for in vitro culture.

2.2. Serum extraction

Fifty milliliters of autologous whole blood was collected by venipuncture and was allowed to sit at room temperature for 30 min in order to clot. The 50 mL tubes were centrifuged at $10{,}000 \times g$ for 30 min. The supernatant was collected and saved as the serum. The pallet was discarded. After centrifugation, serum aliquots were prepared, which were immediately frozen at $-30\ ^{\circ}C$.

2.3. In vitro cell cultures

The chondrocytes were expanded in number using 3 different types of culture media with an initial concentration of 100×10^3 cells in each group. Chondrocytes were plated in 75-cm culture flasks (Falcon, Becton Dickinson, NJ, USA). The nutritional