In Vitro Characterisation of Porous PVA-NOCC Composite Scaffold for Cartilage Tissue Engineering

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Introduction

Cartilage tissue engineering adapted into clinical applications such as autologous chondrocytes implantation (ACI) (Brittberg *et al*, 1993) and the use of mesencyhmal stem cells (MSCs) has emerged as a promising new therapy for the treatment of damaged cartilage. However, it has been found that the success of this therapy relies on the development of a suitable scaffold to provide a template for cell and tissue growth. Scaffolds not only provide the necessary biomechanical strength to withstand joint compressive loading but important to convert the implanted cell-scaffold construct into native cartilage. In an attempt to develop an ideal scaffold or cell carrier, we have synthesised a novel porous three-dimensional composite scaffold based on polyvinyl alcohol (PVA) and N,O-carboxymethylate chitosan (NOCC). Incorporating NOCC with PVA is done to obtain the excellent weight-bearing properties of PVA (Stammen *et al,* 2001) while possessing the biodegradability ability of NOCC. Apart from being both biocompatible and biodegradable, chitosan itself has structural similarity to glycosaminoglycans (GAGs) (Griffon *et al*, 2006) and hyaluronic acid, which are significant components in articular cartilage extracellular matrix (ECM). In the present study, our newly-synthesised PVA-NOCC composite was characterised in vitro to evaluate its potential as a scaffold for future application in cartilage tissue engineering.

Materials and Methods

PVA-117 (M_w=74,000g/mol) was obtained from Kuraray Co. Ltd, Japan whilst NOCC was obtained from Standard and Industrial Research Institute (SIRIM), Malaysia. PVA and NOCC were mixed and the polymer solutions casted into cylindrical moulds. The resultant PVA-NOCC blend was then sterilised and physically cross-linked by irradiation at room temperature, following by frozen at -80°C prior to lyophilisation. The porous composite scaffold was cut into discs (3mm height/5mm diameter). The structure of the scaffold was examined using a scanning electron microscope (SEM). Pore size was measured and analysed. Chondrocytes derived from articular cartilage of New Zealand White rabbits were then seeded at a density of $5X10^6$ on each pre-wetted scaffold, cultured up to 2 and 4 weeks prior to in vitro characterisation. Histological (Safranin-O, immunohistochemistry and DAPI staining), electron microscopy (TEM/SEM) and biochemical analyses (DMMB-GAG and collagen assay) were performed to assess cell-matrix morphology and response.

Results and Discussion

SEM analysis demonstrated that PVA-NOCC scaffold possessed an interconnecting porous structure with pore sizes ranging between 1-200µm. Within 4 weeks, chondrocytes acquired predominantly spherical shapes (Albert *et al*, 1983) and reached confluence on the scaffolds. The cultured chondrocytes adhered extensively to the porous scaffold surfaces and also penetrated interconnecting pores, formed dense colonies and retained their in vivo morphology. Cells were surrounded by distinct fibrillar ECM networks consisting of collagen fibers and proteoglycans. DAPI-stained nuclei were noted to be localised within the scaffold. TEM analysis further revealed that chondrocytes within the construct appeared as healthy and active cartilage cells exhibited normal ultrastructure features, possessed large nuclei with mitochondria distributed intercellularly and abundant ECM secreted by chondrocytes within PVA-NOCC. Histology study confirmed the presence of ECM consisting of proteoglycans and type II collagen. The amount of S-GAG and type II collagen was significantly higher in PVA-NOCC as compared to PVA in both 2- and 4-week culture. The results indicated that PVA-NOCC is superior to PVA alone. PVA-NOCC composite scaffold showed better cell adhesion, proliferation, migration and tissue-integration properties. This porous 3-D composite scaffold also possessed sufficient mechanical properties and demonstrated viscoelastic properties which mimic native cartilage in our previous study (S-Y Lee *et al*, 2009).

Conclusions

The data obtained in this study clearly demonstrates the ability of chondrocytes to maintain phenotypic expression as well as synthesis ECM on our porous PVA-NOCC scaffold. Thus, porous PVA-NOCC is potentially suitable to be used as a scaffold for tissue engineered cartilage in the treatment of articular cartilage defects.

References

1. Griffon DJ, Reza-Sedighi M, Schaeffer DV, Eurell JA and Johson AL. Chitosan scaffold: interconnective pore size and cartilage engineering. Acta Biomaterialia 2006;2: 313-320.
2. Lee S-Y, Pereira BP, Yusof N, Selvaratnam L, Zou Y, Abbas AA and Kamarul T. Unconfined compression properties of a porous poly(vinly alcohol)-chitosan based hydrogel after hydration. Acta Biomaterialia 2009 (in press).