INTRODUCTION

Cell behaviors strongly depend on not only suitable chemistry such as specific bound cell-surface receptor, but also physical properties of scaffold. Nano-sized structure of matrix is important to cell attachment, proliferation and function for tissue repair [1]. The structural design of biomimetic artificial matrix may be the porous 3-D matrix composed of nanometer sized fiber network [2]. However, this nano-fibrous matrix is insufficient to regenerate full thickness defects of tissues such as bone and cartilage, which require the interconnected 3-D architecture of micro-scaled pores. This study attempts to spin chitosan electrically onto the chitosan micro-fibrous sheet and roll the double layered sheet of nano- and micro-fibers. Here we present a novel method to prepare nano- and micro- composite fibrous matrix which provides similar environment of natural extracellular matrix for cartilage regeneration.

MATERIAL AND METHODS

Fabrication of 3-D nano- and micro- composite fibrous matrix: Chitosan micro-fibers were fabricated by wet spinning technique and randomly stacked to form sheets. The composite fibrous matrix was fabricated by electrospinning chitosan at a high voltage on top of the micro-fibrous sheet. The resulting double layered sheet was rolled to produce 3-D fibrous matrix. To study influence of nano-topography to chondrocyte response, chitosan film and various 2-D nano-structured membrane were also fabricated. Scanning electron microscopy (SEM): Morphology of fibrous matrices was observed by SEM at an accelerating voltage of 15Kv. Chondrocyte culture: Bovine articular chondrocytes were isolated by sequential digestion with protease and collagenase. The primary chondrocytes were incubated at 37 °C, 95 °C of humidity and 5 % CO₂ in standard culture medium (DMEM+10 % FBS). Cells reached confluence and then seeded to matrices at a density of 4×10⁶ cells/matrix and to 2-D membranes at a density of 1×10⁵ cells/ml. Cell viability assay: The viable cell number was measured using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2- (4-sulfophenyl)-2H-tetrazolium inner salt (MTS) method using a commercially available kit (Promega, USA). GAG and DNA amount assay. The synthesis GAG was determined by binding to dimethylmethylene blue (DMB) dye and absorbency was measured at 530 nm and 590 nm at each time point. The amount of DNA was determined using indole assay. Total amount of GAG was normalized vs the total amount of DNA. Histological staining: To visualize F-actin, collected matrices were incubated in rodamine labeled phalladin following the manufacturer’s instruction. For the histological assessment of ECM, H & E staining was performed.

RESULTS

The resulting matrix had dual porous architecture containing interconnected webs of nano- and micro-sized fibers. During the electrospinning process of chitosan, nano- and micro-fibers interpenetrated and fused at their contact sites (Fig.1). The nanostructured topography on 2-D membrane enhanced chondrocyte proliferation and phenotype conservation. Fluorescence images of F-actin showed that F-actin reorganized on film on day 3, indicating that the phenotypic change had taken place. Composite fibrous matrix and micro-fibrous matrix with rolling shape were assessed for their scaffolding function by the cultivation of chondrocytes. The proliferation and GAG synthesis by chondrocytes on nano- and micro-composite matrix was significantly greater than that of micro-fibrous matrix (fig. 2, 3). This was confirmed again by H&E and immunohistochemical staining. The cultivation of chondrocytes on nano- and micro-composite matrix resulted in constructing cell clusters and abundant extracellular matrix throughout its entire cross-section (fig. 2).

DISCUSSION

In this study a novel fabrication technique was developed by combining wet spun micro-fibers and electrospun nano-fibers. Nano- and micro-composite fibrous matrix was successfully fabricated as a biomimetic scaffold by electrospinning nano-fibers onto the predefined micro-fibrous sheet for cartilage regeneration. The nano-structured topography enhanced cell attachment and phenotype stability. These findings imply that chondrocytes might recognize nano-structure and this composite matrix provided an environment similar to natural articular cartilage. Therefore, the strategy that incorporate nano-fibers into micro porous framework can be an effective option for cartilage regeneration.