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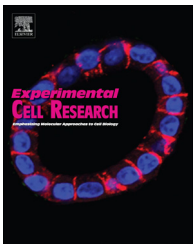

Research Article

Cell–scaffold interaction within engineered tissue

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ABSTRACT

The structure of a tissue engineering scaffold plays an important role in modulating tissue growth. A novel gelatin–chitosan (Gel–Cs) scaffold with a unique structure produced by three-dimensional printing (3DP) technology combining with vacuum freeze-drying has been developed for tissue-engineering applications. The scaffold composed of overall construction, micro-pore, surface morphology, and effective mechanical property. Such a structure meets the essential design criteria of an ideal engineered scaffold. The favorable cell–matrix interaction supports the active biocompatibility of the structure. The structure is capable of supporting cell attachment and proliferation. Cells seeded into this structure tend to maintain phenotypic shape and secreted large amounts of extracellular matrix (ECM) and the cell growth decreased the mechanical properties of scaffold. This novel biodegradable scaffold has potential applications for tissue engineering based upon its unique structure, which acts to support cell growth.

Introduction

An ideal tissue engineered scaffold must have overall constructions, internal structures, surface properties, mechanical properties, and material properties to meet the requirements of host tissue, which are conducive to cell adhesion, proliferation, differentiation, and extracellular matrix formation [1,2]. Although various structures of engineered tissue scaffold have been developed for tissue replacement, the goal of producing a clinically useful tissue scaffold still is far from being realized [3–11]. The final goal of the scaffold is to replace the natural ECM until host cells can repopulate and resynthesize a new natural matrix. To achieve this goal, scaffold should be mechanical stability, which is dependent on the architectural design of the scaffold and the cell–material interactions.

Structural biocompatibility is affected by the physical properties of a scaffold, primarily by its pore, the surface morphology and mechanical property. Pores are essential for tissue formation because they allow the exclusion of metabolites, transportation of oxygen, migration and proliferation of cells, as well as vascularization [12–18]. Porous surface can also improve mechanical interlocking between the implant scaffold and the surrounding tissue, and provide greater mechanical stability at this critical interface [19,20]. Mechanical property and surface morphology, such as substrate hardness, grooves, pillars, ridges, islands and wells to stimulate the cell, transmit signal to ensure the normal phenotype of cell [21–27].

Material biocompatibility is associated with the surface chemistry of the material. The chemical characteristics of a material surface will mediate the adsorption of biologic molecules that regulate cell activities, such as adhesion and migration. The most commonly used natural biopolymers include agarose, collagen, hyaluronan, gelatin, chitosan and alginate. These biodegradable

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materials have a long history of clinical use and currently are used in various tissue engineered applications [28–33].

This paper attempts to combine the advantages of the 3DP technology and vacuum freeze-drying technology to produce scaffold similar to the host tissue. The 3DP based on the layered deposition of fibers can make the scaffold with high reproducible construct and compositional variation, by which the viable scaffold with predefined size can be produced easily [34–36]. Its main merit is that the interconnected porosity of the construct can be easily tailored, which is very important for conductive properties of the construct. Vacuum freeze-drying technology can create micro-morphology on the scaffold by evaporating of the solvent of materials, such as micro-porous and surface morphology, and improve the mechanical property of scaffold.

To explore the applications of this scaffold in tissue engineering, its properties of the structure and its mechanical performance, were characterized and cell activities within the scaffold were investigated. The adipose derived stem cells (ASCs) were seeded into the scaffold in this study. ASCs exhibit advantages including ease of isolation, relative abundance, rapidity of expansion, and multipotency that is independent upon serum source and quality. ASCs possess the capacity of multiple differentiation but primarily osteogenic and chondrogenic differentiation in vivo. ASCs were used in this study because replacement of bone tissue is the possible candidate for future applications of this scaffold.

Materials and methods

Materials

The quantitative Cs was dissolved in acetic acid solution to form a 4% solution. The gel was dissolved in deionized water to form a 20% solution. The above two solutions were mixed into a homogeneous liquid, then, placed to a gel state at room temperature.

Scaffold fabricating

3D scaffold with pre-determined shape and dimension was fabricated with 90° steps between 2 successive layers by extruding the materials on a dish at the room temperature, then, could be fully cross-linked in glutaraldehyde solution followed by vacuum freeze-drying for 53 h. Two groups were made, and one group was made by 3DP as control group, and the other group was made by 3DP/vacuum freeze-drying as experiment group.

Isolation and culture of ASCs

ASCs were isolated from the adipose tissue. Adipose tissue was washed three times with phosphate-buffered saline (PBS, pH 7.4) and treated with 0.075% type I collagenase (Washington Biochemical Corp., USA) at 37 °C for 30 min. Enzymatic activity was neutralized with Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Grand Island, NY), containing 10% fetal bovine serum (FBS, HyClone, USA). The solution was then centrifuged at 1200g for 10 min. The yielding cells were resuspended in the regular medium and plated at 4×10^4 cells/cm^2 in φ100 mm culture dishes (Falcon, USA). The dishes were then maintained at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2 with the media changed twice a week. When having reached 70–80% confluence, cells were passaged and ASCs prior to passage 3 were used in the following study.

Scaffold handling and cell seeding

The scaffold was soaked in alcohol for 1 h, then, washed three times with PBS to replace the alcohol from the scaffold. The excessive solution left within scaffold was removed by extensive suction. ASCs were harvested and suspended in the culture medium at a density of 5.0×10^7 cells/ml. Then, 0.05 ml cell suspensions were respectively seeded into both groups of scaffolds to form cell–scaffold constructs. The cell–scaffold constructs placed in culture dishes, and were added with 8 ml of the growth medium after being incubated for 4 h to allow cell to adhere. The cell–scaffold constructs in media were cultured in vitro, respectively, with the media changed twice a week.

Cell attachment

After being incubated for 7 days, the cell–scaffold constructs were fixed with 4% paraformaldehyde, then, dehydrated. The samples were sputter-coated with gold and observed by scanning electron microscopy (SEM SU1510, Analysis and Testing Center, shanghai university) to determine the adhesion and ECM deposition of ASCs on the scaffold.

Cell proliferation assay

The number of cells on the scaffold was detected by DNA assay respectively at 7, 14 and 21 days later. Briefly, the cell–scaffold constructs collected at different time points were crushed, and then repeated freezing and thawing to release DNA. DNA quantification was performed ($n=9$ per group per time point) using Hoechst 33258 dye (Sigma-Aldrich) following the manufacturer's protocol.

Degradation property

In vitro, degradation of cell–scaffold constructs of two groups was test by monitoring the weight change. Samples were weighted before putting in culture medium, and then incubated in 37° carbon incubator for various periods of time. Two groups of specimens were taken out at the end of each degradation periods, and washed thoroughly with distilled water and then dried the moisture. The control group of scaffold were weighed and the experiment group of scaffold were weighed after vacuum freeze-drying ($n=3$ per group). The degradation rate of scaffold was calculated according to equation:

$$\text{Quality degradation } (\%) = \frac{W_2 - W_1}{W_1} \times 100\%$$

where W_2 and W_1 are the weight of the scaffold after and before degradation, respectively.

Mechanical properties

To explore which factors affected the mechanical property of scaffold, three groups of experiment scaffolds were ready, which were the blank scaffold with no treatment, the scaffolds without cells and the scaffold with cells cultured for 7 days. Took $5\times5\times2$ mm^3 samples from the three groups of experiment scaffolds respectively for testing the mechanical performance, and then pressed them by a testing machine (Instron model 5542, Canton, USA) at room temperature. Samples were compressed