Preparation of Hyaluronan/Collagen II Microsphere by Using a Parallel Electrostatic Field System

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Introduction

Both collagen II and hyaluronan are two major components of the native extracellular matrix (ECM) and tissues, which are responsible for providing the associated tissues with tensile strength, also serve as a structural scaffold for cell adhesion and growth. Many researchers have focused on the preparation and evaluation of man-made ECM, which comprises of these two components, in the form of membrane shape, for chondrocyte cells culture application. In order to increase the versatility of culture applications, a simple and in situ method, by using a parallel high-voltage electrostatic field system, for the preparation of hyaluronan/collagen II microspheres (particulate shape) was presented in this study. Two-step cross-linking treatments (by $FeCl_3$ and 1-ethyl-3-(3-dimethyl aminopropyl) carbodimide, EDC) and 37 °C reconstitution treatment of collagen II molecules were conducted to stabilize the particulate form and further strengthen the obtained microspheres. The results revealed that the hyaluronan/collagen II microspheres exhibited good sphericity and in the range of 679 ± 24 to 486 ± 43 μm in diameter. The mechanical strength and the degradation behavior of these microspheres could also be varied under various defined experimental settings.

Methods

Hyaluronan/collagen II microspheres were produced by extruding mixture droplets, with hyaluronan (0.7 w %, M.w 1.8×10^6 Dalton) and collagen II (3.5 mg/mL, 5 mg/mL and 7 mg/mL), into the cross-linking agent solution. The droplets were generated with the use of a parallel high-voltage electrostatic field system (Fig. 1).

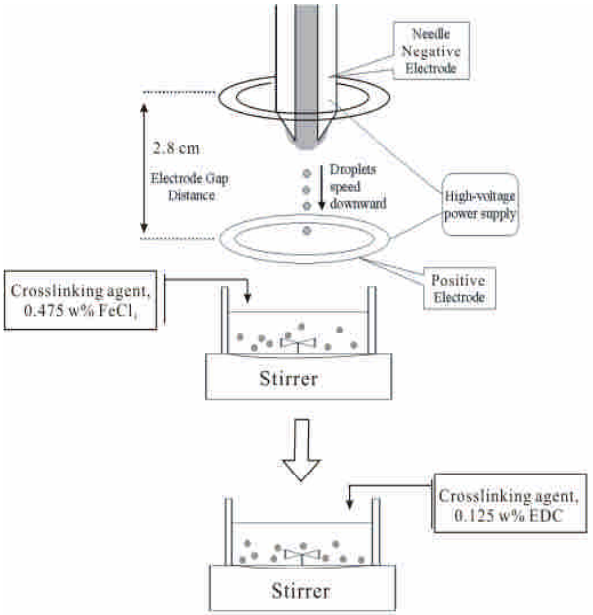

Figure 1 Schematic presentation of hyaluronan/collagen II microspheres preparation.

Results

Optimal electrostatic field system parameter, including two-circle electrode configuration, applied voltage and electrode gap distance for preparing various sizes of microspheres were determined. Various sizes of hyaluronan/collagen II microspheres with a good spherical shape were produced. The size and the related characteristics of hyaluronan/collagen II microspheres are subjected to variations of cross-linking agent treatments and concentrations of hyaluronan /collagen II mixture (Fig. 2). The surface morphology of hyaluronan/collagen II microspheres also changed under $FeCl_3$, $FeCl_3$/DEC cross-linking agents' treatment and collagen II concentration (Fig. 3). More porous structures, which might be beneficial to cell adhering and ingrowth, were created after 37 °C treatment. The microspheres with lower content of collagen II concentration had better mechanical strength after 14-day shaking test.

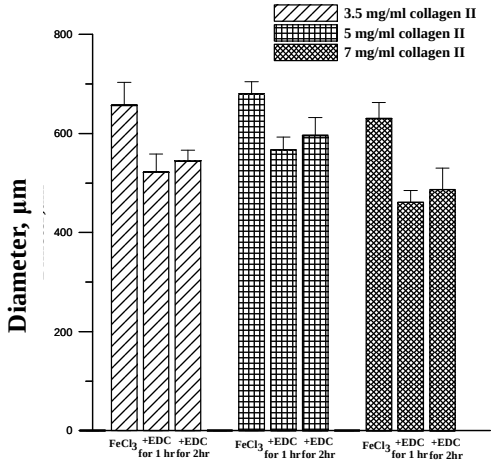

Figure 2 Effects of cross-linking agents' treatment and collagen II concentration on the size of hyaluronan/collagen II microspheres.

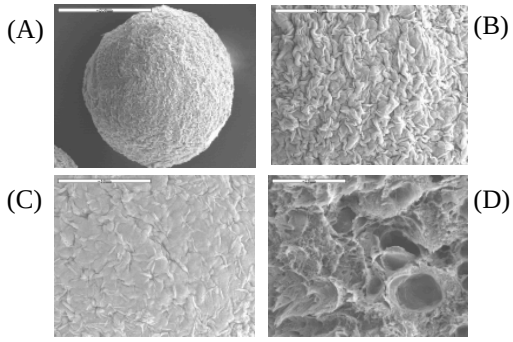

Figure 3 SEM of hyaluronan/collagen II microspheres, (A) $FeCl_3$ crosslinked,×250, (B)$FeCl_3$ crosslinked,×3000, (C)$FeCl_3$/EDC crosslinked, ×3000 and (D) $FeCl_3$/EDC crosslinked/37°C reconstituted,×2000.

Conclusion

To date, we are able to produce different size and surface morphology of hyaluronan/collagen II microspheres, simply by altering the electrostatic field system and the reaction agent treatment. In fact, the applicability of these microspheres as cells carriers is underway in our lab.