Biomaterials 35 (2014) 2607–2616

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Engineering cell matrix interactions in assembled polyelectrolyte fiber hydrogels for mesenchymal stem cell chondrogenesis

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ARTICLE INFO

Article history:
Received 14 November 2013
Accepted 8 December 2013
Available online 2 January 2014

Keywords:
Mesenchymal stem cells
Chondrogenesis
Interfacial polyelectrolyte complexation
Cell–matrix interactions
Cell encapsulation

ABSTRACT

Cell–cell and cell–matrix interactions are important events in directing stem cell chondrogenesis, which can be promoted in matrix microenvironments presenting appropriate ligands. In this study, interfacial polyelectrolyte complexation (IPC) based hydrogels were employed, wherein the unique formation of submicron size fibers facilitated spatial orientation of ligands within such hydrogels. The influence of aligned, collagen type I (Col I) presentation in IPC hydrogel on chondrogenic differentiation of human mesenchymal stem cells (MSC) was investigated. Early morphological dynamics, onset of N-cadherin/β-catenin mediated chondrogenic induction and differentiation were compared between MSCs encapsulated in IPC-Col I and IPC-control (without Col I) hydrogels, and a conventional Col I hydrogel. MSCs in IPC-Col I hydrogel aligned and packed uniformly, resulting in enhanced cell–cell interactions and cellular condensation, facilitating superior chondrogenesis and the generation of mature hyaline neocartilage, with notable downregulation of fibrocartilaginous marker. Inhibition study using function blocking β1-integrin antibodies reversed the aforementioned outcomes, indicating the importance of coupling integrin mediated cell–matrix interactions and N-cadherin/β-catenin mediated downstream signaling events. This study demonstrated the significance of oriented ligand presentation for MSC chondrogenesis, and the importance of facilitating an orderly sequence of differentiation events, initiated by proximal interactions between MSCs and the extracellular matrix for robust neocartilage formation.

1. Introduction

Bone marrow derived mesenchymal stem cells (MSCs) offer an attractive cell source for cartilage tissue engineering due to their autologous origins, ease of extraction from bone marrow, high proliferative capacity *in vitro*, and their ability to be differentiated into chondrocytes [1–3] Yet, cartilage equivalents derived from solely MSCs-based approaches are found to suffer from inferior biochemical content and mechanical strength when compared with native tissue properties [4,5], in many cases, resulting in functionally inferior fibrocartilage formation [6]. To improve the efficiency of stem cell-based tissue engineering for articular cartilage regeneration, there are increasing efforts to develop functionalized scaffolds that mimic aspects of the tissue microenvironment [7–13]. Various adhesive or biochemical cues have been tailored into hydrogels to provide appropriate adhesive cues to improve cell–ECM interactions and facilitate chondrogenic differentiation and cartilage formation [9–13]. However, a significant shortcoming involving such hydrogels is their inability to facilitate robust cell–cell interactions, which are crucial for induction of precartilage condensation and initiation of MSC chondrogenesis [14,15]. Coupled with the low degradability, such hydrogels impede differentiation associated cellular remodeling of local microenvironments [9,16,17], hampering the development of a homogenous, neocartilage tissue *in vitro* [9–11]. Thus establishing a hydrogel platform that can present appropriate biochemical microenvironments in a three dimensional (3D) niche, without compromising

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http://dx.doi.org/10.1016/j.biomaterials.2013.12.008

matrix compliance, cellular distribution and cell–cell interactions is desirable.

To this end, this study reports the use of interfacial polyelectrolyte complexation (IPC) based fibrous hydrogel, as a potential 3D cell encapsulation platform for cartilage tissue engineering. Recent reports have shown chitin–alginate based 3D IPC hydrogels to support the proliferative and differentiation potential of encapsulated MSCs [18–22]. Hydrogel fabrication employing IPC technique has its merits over encapsulation of cells in conventional hydrogels; IPC fibers are formed readily under aqueous conditions at room temperature which makes it amenable for incorporating cells and biochemical cues [23]. Further, the unique formation of submicron size nuclear fibers, owing to the drawing of polyelectrolyte complexes, results in unidirectional orientation of the incorporated cues along such aligned fibers within the hydrogel (Fig. 1B, C). This offers a high degree of spatial resolution of the biomimetic cues with respect to the encapsulated cells within each hydrogel fiber (Fig. 1E, F) [18,23–25]. As mesenchymal progenitors developing into cartilaginous tissues *in vivo* are known to reside in collagen type I rich microenvironments [14,26], IPC hydrogels with collagen type I incorporation (IPC-Col I) were fabricated to study the effect of Col I on MSCs chondrogenesis, in comparison with IPC hydrogel without any cues (IPC-control). A comparative analysis between IPC-Col I and Col I hydrogels was also carried out to appreciate the influence of aligned Col I presentation in IPC fibers. Early cell response and associated molecular events were studied, along with functional analysis of the resulting neocartilage tissue.

2. Materials and methods

2.1. MSC culture and chondrogenic differentiation

MSCs were generated from bone marrow aspirates of consented human donors after obtaining approval from the hospital Institutional Review Board. MSCs were expanded in DMEM supplemented with 10% FBS and 1% (v/v) penicillin–streptomycin. Chondrogenic differentiation was induced in high glucose DMEM supplemented with 10^{-7} M dexamethasone, 1% ITS + premix, 50 μg/mL ascorbic acid, 1 mM sodium pyruvate, 0.4 mM proline and 5 ng/mL of TGF-β3. MSC pellets consisting of 2.5×10^5 cells, formed by 200*g* centrifugation, served as the control to indicate the chondrogenic efficacy of the MSC samples. Cells were differentiated for a 2–3 week period, with medium change every 3 days.

2.2. Encapsulation of MSCs in IPC hydrogel

The fabrication of IPC hydrogel by the assembly of IPC fibers using 1% water soluble chitin (WSC) and 1% sodium alginate solutions was adapted from a previous report [24]. For fiber drawing, 5 μl of each polyelectrolyte solution was dispensed in close proximity and a pipette tip was used to bring the two droplets into contact. The fiber was drawn upwards at a speed of 0.3 mm/s by attaching the pipette tip to a linear motor. For fiber assembly, multiple fibers drawn simultaneously were fused using 0.25% sodium alginate solution and collected on a 2-pronged spooling device [24] and were subsequently removed from the spool and placed in the culture medium. MSCs were suspended in the WSC solution at a density of 1×10^4 cells/μl. Collagen was incorporated into the IPC hydrogel as methylated collagen (MC) in the polycation solution (1% WSC, 0.25% MC). MC was prepared by methylation of type I rat tail collagen as described previously [27].

2.3. Encapsulation of MSCs in collagen hydrogel

A stock solution of rat tail collagen type I in 0.15 M of acetic acid was used to form 3D Col I hydrogels at a final concentration of 2.5 mg/mL in 24 well Transwell® inserts. The Col I mixture containing cells in DMEM was neutralized with 1 N NaOH, prior to being placed in a humidified incubator at 37 °C in 5% CO_2 atmosphere for gelation. The cell encapsulating density, total cell number per hydrogel, and final concentration of Col I concentration were kept the same as in IPC-Col I hydrogel, to facilitate comparative studies.

2.4. Integrin inhibition studies

β1 integrin blocking studies were carried out where MSCs were pretreated with 5 μg/mL of anti-β1 integrin antibody (BD Mab 13, 552828) in serum-free DMEM media for 15 min. Treated cells were then resuspended in appropriate seeding density in media supplemented with 2.5 μg/mL of anti-β1 integrin antibody prior to encapsulation in IPC-Col I hydrogels. Such cell-laden hydrogels were

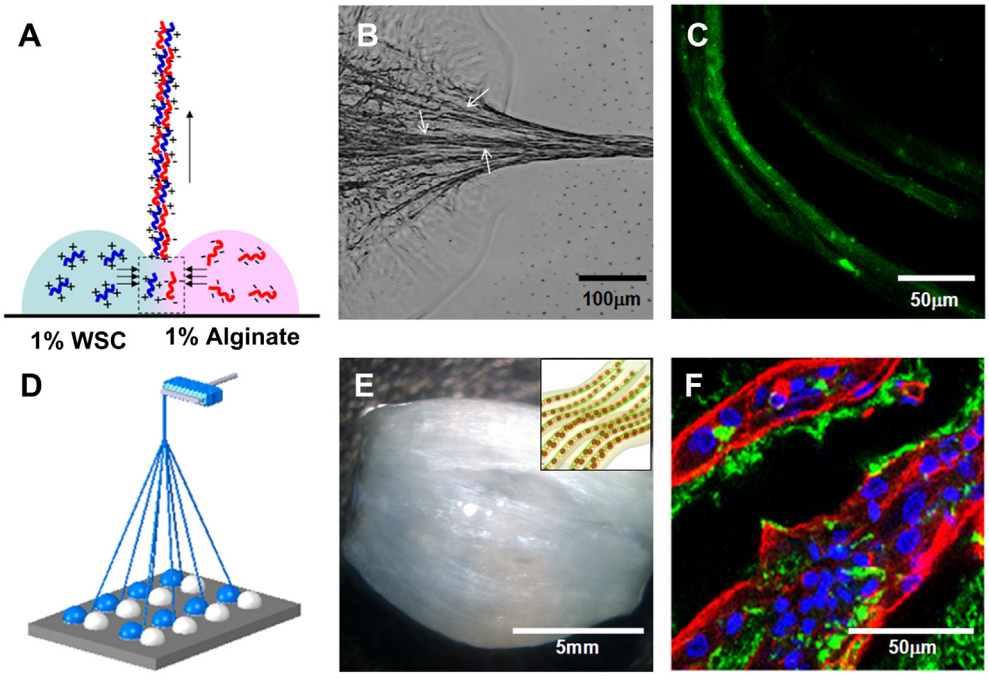

Fig. 1. Fabrication of interfacial polyelectrolyte complexation (IPC) fiber and fiber assembled hydrogel. (A) Schematic illustration of drawing a hydrogel fiber from the interface of the 1% water soluble chitin (WSC) and 1% sodium alginate droplets. (B) Polyelectrolyte complexation and nucleation of IPC fibers as indicated by arrows. (C) Confocal image of FITC-conjugated type I collagen (Col I) incorporated IPC fibers showing Col I distribution. (D) Assembly of multiple fibers by fusion and collection on a spooling device. (E) Fiber assembled hydrogel with illustration of Col I alignment on nuclear fibers and cell distribution in the IPC fibers (insert). (F) Confocal image of FITC-Col I incorporated IPC fibers, with F-actin staining (red) of the encapsulated MSCs showing cell alignment with the FITC-Col I (green). Nucleus was stained by DAPI. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)