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Collagen-based fibrous scaffold for spatial organization of encapsulated and seeded human mesenchymal stem cells

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Abstract

Living tissues consist of groups of cells organized in a controlled manner to perform a specific function. Spatial distribution of cells within a three-dimensional matrix is critical for the success of any tissue-engineering construct. Fibers endowed with cell-encapsulation capability would facilitate the achievement of this objective. Here we report the synthesis of a cell-encapsulated fibrous scaffold by interfacial polyelectrolyte complexation (IPC) of methylated collagen and a synthetic terpolymer. The collagen component was well distributed in the fiber, which had a mean ultimate tensile strength of 244.6 ± 43.0 MPa. Cultured in proliferating medium, human mesenchymal stem cells (hMSCs) encapsulated in the fibers showed higher proliferation rate than those seeded on the scaffold. Gene expression analysis revealed the maintenance of multipotency for both encapsulated and seeded samples up to 7 days as evidenced by Sox 9, CBFA-1, AFP, PPAR γ 2, nestin, GFAP, collagen I, osteopontin and osteonectin genes. Beyond that, seeded hMSCs started to express neuronal-specific genes such as aggrecan and MAP2. The study demonstrates the appeal of IPC for scaffold design in general and the promise of collagen-based hybrid fibers for tissue engineering in particular. It lays the foundation for building fibrous scaffold that permits 3D spatial cellular organization and multi-cellular tissue development.

Keywords

Mesenchymal stem cells; Cell encapsulation; Fibrous scaffold; Collagen; Stem cell tissue engineering; 3D cell patterning

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biomaterials.2008.11.003.

1. Introduction

Spatial organization and distribution of cells within a 3D extracellular matrix are important for controlling cellular functions and neo tissue synthesis [1,2]. To achieve an organized arrangement of cells in a tissue-engineered construct, a scaffold that can facilitate cell remodelling and tissue organization would be attractive. The design and development of scaffolding materials have been constantly evolving, having progressed from an inert mechanical support to a dynamic platform for cellular adhesion, proliferation, differentiation and interaction with the physiological environment [2,3]. Fibrous biomimetic materials are popular candidates because they provide a 3D microenvironment with high surface area-to-volume ratio, offer the potential of presenting biological cues in a temporally and spatially controlled manner, and impart a controlled porous architecture for efficient waste/nutrient exchange and cell migration [4–9].

Optimal tissue development requires infiltration of cells into the scaffold, which in turn necessitates a macroporous structure with interconnected pores diameters of at least 10 μm [10–12]. Alternatively, seeded cells can migrate into the interior of the scaffold by either enzymatically degrading or displacing individual fibers, but this requires an extended culture period and appropriate chemotactic factors present within the scaffold [12,13]. As the scaffold thickness increases and the pore size decreases, the problem of hindered cell infiltration becomes significant. Various strategies have been proposed, and they are based on a common hypothesis that scaffolds embedded with cells in a controlled spatial distribution can address the current problem of limited cell infiltration and achieve a highly cellularized tissue construct. To this end, mesenchymal stem cells and smooth muscle cells have been encapsulated in photosensitive hydrogels [14,15]; fibroblasts and endothelial cells sprayed between gels [16]; smooth muscle cells sprayed between layers of electrospun fibrous mats [17]; and cells incorporated into fibers via co-axial electrospinning [18]. Cells have also been printed onto scaffolds using modified ink-jet print heads [19,20]. Although the reported studies have made significant progress in creating highly biofunctional scaffolds, the said processes are complex and often detrimental to cell viability. Hence a milder and simpler technique to incorporate cells into a 3D scaffold would be desirable. A recently developed technique is interfacial polyelectrolyte complexation (IPC) [4,6].

Based on electrostatic interaction of oppositely charged poly-electrolytes, IPC can produce stable fibers under aqueous and room temperature conditions for scaffold construction [4]. Unlike many current scaffold fabrication techniques [15,21,22] which involve the use of volatile organic solvents and cytotoxic photocrosslinkers that may be detrimental to the bioactivity of biologics [23] and viability of encapsulated cells, IPC is amenable to encapsulation of proteins [4], cells [6] and DNA [24] into the fibers. Encapsulation of cells with IPC technique has additional advantages over encapsulation of cells in gels; the porous architecture allows efficient nutrient/waste exchange and in essence 3D cell patterning.

We have previously reported the construction of alginate–chitosan PEC scaffold for cell encapsulation [6]. However, weak fiber mechanical properties, poor cellular adhesion and uneven cell distribution were observed. Hypothesizing that collagen could be an attractive cation to form PEC fibers, we study the complexation of methylated collagen with a custom-synthesized anionic terpolymer to produce a hybrid fibrous scaffold that might exploit the favorable biological properties of collagen and the tunable physical properties of a synthetic polymer.

Scaffolds made of synthetic polymers are versatile with tunable physical properties, but typically lack cell recognition signals for effective cell attachment [10]. Natural polymers such as collagen, elastin and glycoaminoglycans possess biological cues for cellular

interaction but often lack the appropriate mechanical properties required as structural biomaterials for tissue regeneration [8]. A hybridization of both polymer types by IPC may produce fibers with the desired complementary properties. In this study, we examined the mechanical properties, surface morphology and collagen distribution in the collagen–terpolymer PEC fibers. We also compared the proliferation and differentiation of hMSCs seeded on (hMSC-seed) and encapsulated (hMSC-encap) within the fibrous scaffolds for three weeks in proliferating medium.

2. Materials and methods

2.1. Materials

Methyl methacrylate (MMA), hydroxyethyl methacrylate (HEMA) and meth-acrylic acid (MAA) monomers and 2,2'-azobisisobutyronitrile (AIBN) were purchased from Sigma–Aldrich Chemicals Ltd. (Singapore). The monomers were purified by vacuum distillation and AIBN was recrystallized in ethanol before use. Collagen Type I (Nutragen, 100 mL) was purchased from Nutacon, Netherlands.

2.2. Terpolymer synthesis

The terpolymer was synthesized in 100 mL of isopropanol via radical polymerization at 60 °C. MMA, HEMA and MAA monomers were charged into a 250 mL round bottom flask at a molar ratio of 25:25:50 [25] and 0.25 wt% of AIBN was used as an initiator. The reaction mixture was cooled for 5 min in an ice bath, purged with argon for another 5 min before it was immersed in a hot silicone oil bath. The polymerization process was carried out for 16 h, after which the mixture was cooled rapidly under running water. The terpolymer was precipitated twice in 1 L of hexane and vacuum dried. The dried terpolymer was subsequently dissolved in 1 M NaOH to convert the carboxyl acid groups to sodium carboxylate, dialyzed against deionized water for 24 h or until the external reservoir reached a pH of 5.5 and the final anionic terpolymer product was recovered by lyophilization.

2.3. Collagen methylation

12 mL of collagen (6 mg/mL in 0.012 N HCl) was precipitated in 400 mL of acetone and redissolved in 200 mL of methanol containing 0.1 M HCl. The mixture was stirred for 2 days at room temperature before it was dialyzed against deionized water (Spectrapor MWCO = 3500) until the external reservoir reached a pH of 5.5. The methylated collagen was lyophilized and stored at –80 °C before use.

2.4. Terpolymer–collagen fiber formation

Terpolymer–collagen fibers were drawn out of the interface between terpolymer and methylated collagen droplets. The terpolymer and methylated collagen concentrations used were 5.5 mg/mL and 5.0 mg/mL respectively. 5 µL of terpolymer and methylated collagen droplets were placed 1 mm apart on a Petri dish and a pair of tweezers was used to bring the 2 droplets together to draw a fiber. The fiber was attached to one of the supporting rods on a motorized roller and the fiber was drawn continuously at a rate of 10 mm/s until it was terminated by the depletion of polyelectrolyte droplets. The fibers collected on the roller were air-dried before they were removed and observed under a scanning electron microscope (SEM, FEI Quanta 200F, USA).

2.5. Fiber characterization

2.5.1. Mechanical property measurement of fibers—The tensile properties of the fibers were performed according to ASTM D 3822 using a nanotensile testing system (Nano Bionix System, MTS, USA). The sample preparation for the tensile test was described

previously [26]. Briefly, 10 single strands of terpolymer–collagen fiber were individually mounted onto a cardboard frame and secured with adhesive tapes at the ends before the frame was mounted on the nanotensile tester. Each fiber has a gauge length of 10 mm. The sides of the frame were snipped off before the fiber was subjected to uniaxial tensile loading at a strain rate of $3.7 \times 10^{-2} \text{ s}^{-1}$ until they break.

2.5.2. AFM imaging—Atomic force microscope (AFM) (Dimension 3100, Digital Instruments, USA) was used to study the fiber surface morphology. Fibers were collected on a mica surface, dried in a desiccator for a day before imaging in tapping mode. Silicon nitride tips with resonance frequencies of 279–300 kHz were used for this study.

2.5.3. Quantum dot labelling of collagen—Biotinylated anti-Collagen Type I (Rabbit) antibody (Rockland Immunochemicals, Inc. USA) was added to the methylated collagen solution in a dilution ratio of 1:3000. The mixture was incubated at room temperature for 3 h and dialyzed against deionized water. Streptavidin-conjugated quantum dot 605 nm (Invitrogen, Singapore) was added at a dilution of 1:50 and incubated for 10 min in the dark. 5 μL of the quantum dot labelled methylated collagen and 5 μL of terpolymer were used to draw the fibers. The fibers were collected on glass coverslips, washed with PBS and mounted onto glass slides for confocal imaging.

2.6. hMSC culture studies

hMSCs were purchased from Cambrex (Poietics; Lonza, Switzerland), cultured and expanded in mesenchymal stem cell growth medium (MSCGM™). The cells used in the experiments were between passages 4 and 7.

2.6.1. Seeding hMSCs on fibers—Dried PEC fibers were immersed in 70% ethanol for 30 min before 3 h of UV irradiation. They were washed 3 times with sterile PBS and placed in 24 well culture plates. 100 μL of cell suspension containing of 10^4 cells hMSCs was added to the top of the fibers and after 30 min of cell seeding, fresh MSCGM was added to the culture well. The cultures were incubated at 37 °C with 5% CO₂.

2.6.2. Encapsulating hMSCs in fibers—hMSCs cultured in 25 T flasks were trypsinized and washed with PBS before adding to 100 μL of methylated collagen to make a cell suspension of 3×10^5 cells/mL. 5 μL of methylated collagen containing cells were placed on a sterile Petri dish next to 5 μL of terpolymer solution. A pair of tweezers was used to bring the 2 droplets together and draw a cell-encapsulated fiber. The fiber was collected on a glass coverslip and washed with PBS before immersing in cell culture medium immediately. This was repeated until 20 cell-encapsulated fibers were collected and the samples were transferred from the glass coverslip into a 6-well Transwell plate and incubated at 37 °C with 5% CO₂.

2.7. Cell viability and proliferation studies

The viability and proliferation of hMSCs-seed and hMSCs-encap were verified by Alamar Blue and live/dead cell assay (Molecular Probes and Sigma–Aldrich, Singapore). 40 μL of Alamar Blue solution was added to 400 μL cell culture medium and the samples were incubated for 3.5 h. The medium was removed and the fluorescence intensity readings were taken at excitation and emission wavelengths of 544 nm and 590 nm respectively. For live/dead assay, CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate) and propidium iodide (PI) were used. In brief, the cells were incubated in 300 μL of cell culture medium containing 0.02 mM CMFDA for 45 min at 37 °C, washed with PBS before incubating in 300 μL of 0.05 mg/mL PI for 5 min. The samples were washed twice in PBS and viewed under fluorescence microscope immediately.

2.8. Cytoskeletal staining

The samples were fixed with 4% paraformaldehyde for 20 min, permeabilized in 0.1% Triton X-100 for 20 min and blocked with 1% BSA for 1 h. For F-actin and nuclei staining, the samples were incubated with TRITC phalloidin (dilution 1:500) and DAPI (1:2500) for 20 min at room temperature before they were mounted and viewed under a confocal fluorescence microscope.

2.9. Reverse transcriptase PCR studies of hMSCs

The samples were stabilized with RNeasy lysis reagent (Qiagen, Singapore) and homogenized with QIAshredder. The RNA was isolated using RNeasy Minikit (Qiagen, Singapore) and synthesis and amplification of cDNA were carried out with Qiagen One-Step RT-PCR kit with the primers (1st Base, Singapore) listed in Supplementary data and the annealing temperature was 60 °C. Human β -actin was used as a housekeeping gene. 33 cycles of PCR were carried out on an MJ Research PTC 100 Thermal Cycler and the PCR products were resolved in an agarose gel (1.2%) in 1× TAE buffer (60 V at 10 cm).

3. Results

3.1. Terpolymer–collagen fibers

The terpolymer (MMA–HEMA–MAA) was converted to an anionic polyelectrolyte by adding 1 M NaOH at the end of the polymerization reaction. Sodium hydroxide converted the sodium carboxylic acid groups in methacrylic acid (MAA) to carboxylic acid sodium salt which increased the terpolymer solubility in aqueous solution and gave the terpolymer a net negative charge when dissolved at neutral pH. The native collagen was converted to a cationic polyelectrolyte through methylation. The carboxylic groups in collagen were esterified which disrupted the positive and negative charge equilibrium and resulted in a net positive charge when dissolved in aqueous solution.

When both polyelectrolytes in the form of droplets were brought together, a fiber was drawn at the interface through the mechanism of interfacial polyelectrolyte complexation (IPC) [27]. This mechanism refers to the formation of an insoluble complex at the interface when the polyelectrolyte charges were neutralized upon contact. The insoluble complex was drawn away from the interface in the form of a fiber and the process terminates when the polyelectrolyte droplets deplete.

Beads were present in the fibers during fiber formation and they were spaced at regular intervals along the fiber length. When the fibers were left to dry, the beads would shrink and form slight protuberances. This observation was also reported by Wan et al. when alginate–chitosan polyelectrolyte complex fibers were made [27]. Fig. 1 shows the experimental setup for fiber fabrication and the SEM image of the fibers.

3.2. Mechanical properties of fibers

The uniaxial tensile test results of the fibers are presented in Table 1. The methylated collagen concentrations of 3 mg/mL and 5 mg/mL were used to obtain 2 sets of fibers for the tests. It was observed that at concentrations above 5 mg/mL and below 3 mg/mL, the methylated collagen solutions were either too viscous or too diluted for fiber formation respectively. The ultimate tensile strength of fibers made with 5 mg/mL methylated collagen was 244.6 ± 43.0 MPa and the strain at failure was $24.4 \pm 4.1\%$. When the methylated collagen concentration was reduced to 3 mg/mL, the ultimate tensile strength was reduced to 212.9 ± 42.0 MPa and the strain at failure was $19.7 \pm 3.0\%$. Uniaxial tensile tests were performed to investigate the changes in the PEC fiber mechanical properties at both upper

and lower methylated collagen concentration limits permissible for fiber formation. No significant difference could be detected within this narrow concentration range.

Determination of the mechanical properties of the hydrated PEC fibers was attempted but without success. The hydrated PEC fibers were too delicate for the clamps to hold and the fibers dried up quickly before a measurement could be completed. Spraying PBS on the PEC fibers was attempted but the wetness of the fibers was not homogeneous, especially at the clamp areas.

3.3. AFM imaging and quantum dot labelling of collagen

3.3.1. Fiber diameter measurement—The AFM height image of the fiber is shown in Fig. 2A. The fiber was collected on a flat mica substrate and the fiber diameter was determined by section analysis of the height image. Fig. 2B shows the amplitude image of the fiber in which the topographical details of the fiber surface can be observed clearly. The fiber surface was not smooth and has ridges and valleys that aligned with the fiber axis. The section analysis of the fiber image across the indicated line and red arrows is shown in Fig. 2C where the peak represents the fiber (see Appendix). The mean fiber diameter was $3.31 \pm 0.11 \mu\text{m}$ based on 30 measurements.

3.3.2. Fiber morphology and distribution of collagen in fibers—AFM height and amplitude images of the fiber bead are shown in Fig. 2D and E respectively. For observation of fiber beads, the fibers were collected just before they were completely dried. The AFM images showed the presence of sub-micron fibrils “fanning out” from the main fiber to form the bead region. A section analysis of the bead in Fig. 2F shows the height difference between the main fiber and the bead region (indicated by the red and green arrows respectively) (see Appendix). The bead region is flatter with the fibrils spreading over a larger area compared to the core fiber.

The collagen distribution in the fiber was determined by labelling the collagen proteins with quantum dots. The confocal images showed that the collagen distribution was uniform in the core fiber region. In contrast, collagen distribution was heterogeneous in the bead regions and it was concentrated mainly at the fiber core. At higher magnification of the core fiber, it was clear that collagen was uniformly distributed within each of the sub-micron fibrils, as shown in Fig. 2G and H.

Fig. 3 shows the AFM scans of the main fiber surface which reveal sub-micron sized fibrils aligned along the fiber axis. Fig. 3A and D shows the AFM height images of the fiber generated when the AFM tip scanned across the fiber surface to reveal the fiber topographical details of the fiber. Fig. 3B and E shows the AFM amplitude images, which are more sensitive to the difference in height topographies of the fiber surface and hence provide a better resolution of the sub-micron fibrils details. The AFM phase images in Fig. 3C and F showed that the fiber has a heterogeneous composition and that the sub-micron fibrils are mainly made up of distinct material phases.

The fibers were not fixed before dehydration because we attempted to compare the fiber surface morphology in dry and wet conditions. However, the hydrated fibers became sticky during AFM imaging and thus the study was unsuccessful.

3.4. Cell encapsulation, viability and proliferation in scaffolds

The hMSC concentration used for cell encapsulation has to be optimized to ensure that fiber formation was not compromised and there were sufficient amount of cells encapsulated within the fibrous scaffolds. We found that 2×10^6 cells/mL was the maximum cell

concentration we could encapsulate before fiber formation ceased. However after 1 week of culture, most of the hMSC-encap had migrated out of the fibers and fiber fragments were observed at the bottom of the culture plate. Fibers without hMSCs were used as controls and no fiber fragments were observed for the same time point, indicating that the fiber fragments were not caused by fiber degradation (data not shown). The hMSC concentration was further optimized to 3×10^5 cells/mL to minimise overcrowding and fiber fragmentation.

The live/dead assay results in Fig. 4A–F show that the hMSCs remained viable in all the scaffolds after 21 days. hMSCs-encap were rounded while hMSCs-seed adhered and elongated along the fiber axis. The viability and proliferation of hMSCs-encap and hMSCs-seed were examined with Alamar blue assay (Fig. 4G). The absorbance values obtained at days 14 and 21 were normalized with those at day 7 to observe the rate of proliferation in hMSCs-encap and hMSCs-seed. hMSCs-encap increased about 2.5-fold at day 14 and 17-fold at day 21, while hMSCs-seed increased about 1.5- and 5-fold at days 14 and 21 respectively. The results show that the cell-encapsulation process is not detrimental to the cell viability and that the PEC fiber supports the proliferation of the encapsulated cells for at least 21 days.

3.5. Cytoskeletal organization of cells in scaffolds

The cytoskeletal organization of hMSCs-encap and hMSCs-seed is shown in Fig. 5. hMSCs-encap were mostly spherical or elliptical (Fig. 5i, A–C) and tiny actin filaments were observed to be extending out of some cells (Fig. 5ii, A). hMSCs-encap were observed on different z planes of the scaffolds, demonstrating that our fibrous scaffolds have cells well distributed within its architecture. (Fig. 5ii, B).

hMSCs-seed elongated with the fibers are shown in Fig. 5i(D–F). The actin fibers in the cells aligned with the PEC fiber direction and the cells were observed to elongate and make cell–cell contact with one another. Fig. 5ii, C shows 3 hMSCs seeded on a fiber strand elongating and contacting one other while an hMSC seeded on a perpendicular fiber (indicated by white arrow) was observed to elongate and establish contact with these cells. A 3D reconstruction of the confocal z-stack images in Fig. 5ii, D shows seeded hMSCs elongating and following the contours and topography of the fibers.

3.6. mRNA expression studies

Fig. 6 shows the expression of neural, hepatogenic, chondrogenic, osteogenic and adipogenic markers in both hMSC-encapsulated and hMSC-seeded scaffolds between days 7 and 21. hMSCs cultured on tissue culture flasks in MSC growth medium were used as control. All samples expressed Sox 9, CBFA-1, AFP, PPAR γ 2, nestin, GFAP, collagen I, osteopontin and osteonectin. HNF4 α , albumin, osteocalcin, collagen II, collagen X and leptin expressions were absent in all samples. Aggrecan and MAP2 expression were observed in hMSCs-seed at days 14 and 21. A faint band for Noggin was detected in hMSC control and the expression was upregulated in both encapsulated and seeded hMSC samples at day 14 and day 21.

4. Discussion

4.1. Fiber characterization

Polymers from synthetic and natural origins have their own advantages and disadvantages when used as tissue scaffolding materials. The PEC fibrous scaffold is designed to take advantage of the complementary merits of these two classes of biomaterials. Interfacial polyelectrolyte complexation has been used to produce polyion complex fibers like poly(lysine)–gellan [28] and water-soluble chitin–alginate [4] and we have previously

applied this concept to encapsulate different biologics, including cells, using the alginate–chitin polyelectrolyte pair [4,6]. However, these alginate–chitin PEC fibers were mechanically weak and suboptimal for cell adhesion and proliferation. Hypothesizing that inclusion of collagen would enhance cellular interaction; we seek to develop improved PEC fibers in this study and a custom-designed polyanion renders this collagen-based PEC fiber design possible.

The MMA–HEMA–MMA terpolymer was chosen as the anionic polyelectrolyte because of its tunable material properties. Each monomer was considered for a purpose: HEMA was incorporated to provide hydrophilicity and water absorption, MMA the mechanical strength and MAA the net negative charges needed to complex with methylated collagen [29]. Collagen was used to provide the integrin receptors for cell adhesion and proliferation. The methylation process provided the net positive charges for IPC. The resultant polyelectrolyte complex fibers possessed higher mechanical tensile strength and failure strain compared to alginate–chitosan polyelectrolyte complex fibers [6,30].

Quantum dot labelling of collagen confirmed the presence and distribution of collagen in the fibers. Factors contributing to bead formation in polyelectrolyte complex fibers are unclear, although there have been hypotheses that charge instability and presence of aggregates at the fiber forming interface [27] prevent the fibrils from coalescing together to form a fiber. The beads collapsed into slight bumps when the fibers were left to dry, thus suggesting that the gel in the beads could have been the excess aqueous solvents that were drawn up into the fibers during fabrication. The presence of cells within a fiber would alter the fiber properties significantly and it would be of interest to investigate the change in fiber strength with the amount of cells encapsulated within it.

4.2. hMSCs-encap and hMSCs-seed behaviour and interaction with PEC fibers

As anchorage-dependent cells, the viability of hMSC drops considerably when encapsulated in a hydrogel that does not present any biological cues for adhesion [8]. In this study, live/dead staining and proliferation assays demonstrate the capability of the PEC fibers to support the proliferation of hMSCs-encap for at least 21 days. The high cell number observed in hMSCs-encap samples might be attributed to the presence of collagen in PEC fibers which provide the integrin sites needed for cellular attachment.

Cell encapsulation by IPC is simple and rapid. When both oppositely charged polyelectrolytes are in contact at the interface, the formation of the insoluble complex and up-drawing of it as a nascent fiber cause a decrease in fluid density at the interface area. This decrease led to the diffusion and convection of more polyelectrolytes and cells towards the interface, where little “whirl-pools” were generated and observed. The cells were “trapped” and encapsulated into the fiber and the “whirl-pools” continued to push more polyelectrolyte and cells towards the interface area until the fiber terminated.

There is a limit on cell density achievable in the PEC fibers. The ratio of cells to PEC fibers formed instantaneously at the interface is an important parameter to optimize. When the cell concentration exceeds 2×10^6 cells/mL, the presence of cells at the interface area between the polyelectrolytes would prevent fiber formation. At lower cell concentrations (less than 5×10^3 cells/mL), the cell-encapsulation efficiency suffers. Thus, achieving an optimum condition where there are substantial amount of cells encapsulated in the fibers without compromising fiber formation is necessary. The morphology adopted by hMSCs-encap and hMSCs-seed is clearly distinct. hMSC-encap are spherical or elliptical in shape and the tiny actin filament extensions observed suggest that these cells are able to establish focal adhesions within the fibers and extend their actin filaments along the fiber axis. In

comparison, hMSCs-seed adhere well onto the fibers, and the actin filaments are more organized, with a propensity of orienting along the fiber axis.

4.3. mRNA expressions

hMSCs have the potential to differentiate to various lineages [31]. As hMSCs adopted different morphologies when encapsulated within and seeded onto PEC fibers, the possibility of the cells undergoing differentiation under such conditions was investigated. The expression of early markers of chondrogenesis, osteogenesis, adipogenesis and neurogenesis suggested that hMSCs maintained their pluripotent differentiation potential for at least 7 days, despite the significant morphological changes. By 14 days, the hMSC-seed expressed genes that were specific to neuronal lineage, as evidenced by the appearance of MAP2, a dendrite-specific microtubule-associated protein found specifically in dendritic branching neurons [32]. This gene is thought to be involved in microtubule assembly, an essential step in neuro-genesis. MAP 2 expression was downregulated in hMSC-encap samples compared to the control, but in contrast upregulated in hMSCs-seed samples. By day 21, MAP 2 expression in hMSCs-seed samples was 3-fold higher compared to the control (see Supplementary data).

The trend observed is hypothesized to be related to the morphology adopted by the hMSC in different culture configurations. On TCPS control, the hMSC harvested after 7 days of expansion showed a spindle shape at near confluent state and a low MAP2 expression. Seeded on the PEC fibers, hMSC adopted a more elongated morphology than the control with an occasional dendritic appearance, and hence an upregulation of MAP2 consistent with previous results observed on nanogratings [33]. Encapsulated hMSC did not exhibit any dendritic cell features like hMSCs-seed and thus MAP2 expression was downregulated in these samples.

Noggin is an antagonist to bone morphogenetic proteins and promotes neurogenesis [32]. This gene was upregulated in both hMSCs-encap and hMSCs-seed by day 14. Aggrecan expression was also observed in seeded hMSCs at days 14 and 21. The aggrecan gene studied belongs to the aggrecan/versican proteoglycan family found abundant in the cartilaginous tissue and central nervous system (CNS). It is expressed in glial precursors and appears to play an important role in early neuronal development [34,35]. Although the relation between aggrecan and neuronal development is still controversial, the expression of other genes such as Nestin, GFAP and Noggin seems to suggest that hMSCs-seed have the inclination to undergo neuronal differentiation.

The presence of multiple lineage marker expressions in the hMSCs indicates that the scaffold itself does not direct the hMSCs into a particular lineage and hence it is a versatile system for different types of tissues. This would be particularly useful where different layers of scaffolds co-encapsulated with hMSCs and specific inductive factors can drive differentiation accordingly to form a complex tissue engineering construct.

The IPC technique of encapsulating cells in fibers is to date the only technique that allows 3D patterning of stem cells in a construct. Where uniform cell distribution in a macroporous scaffold is usually a challenge, the PEC fibrous scaffold bypasses this issue since the cells are incorporated into each fiber strands and the semipermeable porous nature of the scaffold can facilitate waste/nutrient exchange. A highly cellularised construct can be achieved by increasing the layers of cell-encapsulated PEC fibers. Finally, this cell-encapsulation technology can also be extended to 3D co-culture studies where one cell type is encapsulated to serve as a stromal layer to provide support for a different cell type seeded on the fibers.

5. Conclusion

Fabrication of cell-encapsulated PEC fibers permits the 3D patterning of cells within a matrix, a first step towards the creation of a complex biological construct. The collagen-based composite PEC fibrous scaffold and the mild processing conditions support the viability and proliferation of cells encapsulated within the fibers. The mechanical properties of these fibers are superior compared to alginate–chitosan PEC fibers. Each individual PEC fiber is a bundle of sub-micron collagen-rich fibrils that appear to provide topo-graphical guidance for hMSCs-encap to extend its actin filaments and hMSCs-seed to elongate and establish contact with one another. The mRNA expression studies indicate the conservation of hMSC pluripotency within the first 7 days of culture and the inclination of hMSCs-seed to express neuronal-specific markers after prolonged culture.

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Appendix

Figures with essential colour discrimination. The majority of figures in this article are difficult to interpret in black and white. The full colour images can be found in the online version, at doi: 10.1016/j.biomaterials.2008.11.003.

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Fig. 1. Terpolymer–collagen polyelectrolyte complex fibers formed by IPC. (A) Schematic diagram showing the drawing of fiber from the polyelectrolytes interface. (B) Spun fibers collected and dried on the motorized roller. The dried fiber meshes are indicated by the white arrow. (C) SEM micrograph of the dried fibers.

Fig. 2. AFM height and amplitude images and section analysis of the main fiber (A)–(C) and bead region (D)–(F) respectively. Quantum dot labelling of collagen distribution in (G) bead region; bar = 100 μm , and (H) in the main fiber; bar = 1 μm .

Fig. 3.

(A)–(C) 4 mm and (D)–(F) 1.57 μm AFM height, amplitude and phase images of collagen–terpolymer fiber, respectively. The AFM height scale bars are presented at the extreme left. The height (A, D) and amplitude images (B, E) show the topographical details of the fibrils of the main fiber and the phase images (C, F) show the material composition variations of the fiber.

Fig. 4. Live/dead assay of encapsulated (A)–(C) and seeded hMSCs (D–F) at days 7, 14 and 21. Bar = 100 μm; (G) Alamar blue viability and proliferation assay of the hMSCs from day 7 to day 21. ▨ denotes hMSCs seeded onto PEC fibers and ■ denotes hMSCs encapsulated in PEC fibers.

Fig. 5.

Cell cytoskeletal organization of (i) A–C hMSCs encapsulated in PEC fibers at days 7, 14, and 21. Bar = 20 μm . (i) D–F hMSCs seeded on PEC fibers at days 7, 14 and 21. Bar = 50 μm . (ii) A shows the actin filament extensions in encapsulated hMSCs (indicated by white arrows). Bar = 20 μm . (ii) B and D show the 3D images of hMSCs encapsulated in and seeded on PEC fibers, respectively. (ii) C shows hMSCs elongating and establishing cell–cell contact with one another. Bar = 50 μm . Actin is stained red and the cell nucleus blue (see Appendix).

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Table 1

Mechanical properties of terpolymer–collagen fibers.

Fiber composition	Tensile strength (MPa)	Strain at failure (%)
Terpolymer 0.55 wt%–collagen 5 mg/mL	244.6 ± 43.0	24.4 ± 4.1
Terpolymer 0.55 wt%–collagen 3 mg/mL	212.9 ± 42.0	19.7 ± 3.0