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Human mesenchymal stem cell culture on heparin-based hydrogels and the modulation of interactions by gel elasticity and heparin amount

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ABSTRACT

Human adipose-derived stem cells (hADSCs) are a promising cell source for tissue engineering and regenerative medicine with no ethnical issue and easy access of large quantities. Conventional surfaces for hADSC culture, such as tissue culture plates (TCPs), do not provide optimal environmental cues, leading to limited expansion, loss of pluripotency and undesirable differentiation of stem cells. The present study demonstrated that heparin-based hydrogels without additional modification provided an excellent surface for adhesion and proliferation of hADSCs, which were further tunable by both the amount of heparin (in a positive way) and the elasticity of hydrogel (in a negative way). The optimized heparin-based hydrogel could selectively modulate the adhesion of hADSCs and human bone marrow stem cells (but not all kinds of cells), and resulted in a significant increase in cell proliferation compared to TCP. Furthermore, in terms of the maintenance of pluripotency and specific differentiation, heparin-based hydrogel was much superior to TCP. The selective binding and proliferation of human mesenchymal stem cells on heparin-based hydrogel over other hydrogels were largely mediated by integrin β1 and selectin, and these superior characteristics were observed regardless of the presence of serum proteins in the culture medium. Consequently, heparin-based hydrogel could be a powerful platform for cultivation of mesenchymal stem cells in various applications.

1. Introduction

Stem cells, with the pluripotency to differentiate into diverse cell types as well as the capacity of self-renewal, are considered as a highly promising cell source for tissue engineering and regenerative medicine [1]. In recent years, due to ethical concerns regarding embryonic stem cells, the use of adult stem cells has been the focus of attention. In particular, human adipose-derived stem cells (hADSCs) have received increased attention since they are easily accessible in large amounts and possess multilineage differentiation potential [2]. The successful utilization of hADSCs for regenerative and tissue engineering applications is strongly dependent on the ability to regulate the cellular fate, including cell morphology, adhesion, proliferation and differentiation, during the culture of these cells on a matrix. To implement hADSCs as a cell source, critical points to be considered as a culture substrate are to improve the cellular adhesion and proliferation, as well as to guide the differentiation lineage. A conventional microenvironment for hADSCs culture comprises tissue culture polystyrene plate (TCP) surfaces, but the culture of hADSCs on TCP normally leads to a limited proliferative capacity, incomplete pluripotency and uncontrollable differentiation of the cells due to non-specific and non-biological interactions associated with the TCP [3,4]. Therefore, an optimal tissue-engineered matrix that can regulate cellular fate is critically important for the maximization of the therapeutic potential of hADSCs. To date, various biomaterials possessing appropriate physical properties have been designed to provide the proper microenvironment to control cellular fate, mostly for embryonic stem cells [5–9], whether by the incorporation of peptides [5,6,8], the immobilization of hyaluronic acids [7] or the modification of TCP by ultraviolet (UV) irradiation [9].

A number of hydrogels have also been employed as biomimetic culture platforms for stem cells [10]. Hydrogels based on natural polymers could provide a proper microenvironment for cell cultivation, but these are difficult to manipulate (e.g. control chemical/mechanical properties) and potentially immunogenic [11,12]. On the other hand, synthetic polymer-based hydrogels can be easily manipulated to create well-defined materials, but many of them have only limited interaction with cells and therefore are not applicable for subsequent cultivation of stem cells [13,14]. To provide a complementary approach combining potentials of these two kinds of polymers, we previously developed a bioactive heparin-based hydrogel by reacting thiolated heparin (Hep-SH) and poly(ethylene glycol) diacrylate (PEG-DA) [15,16]. The heparin-based hydrogel was an effective cell culture platform that presents biochemical

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and biophysical properties suitable for cultivation of hepatocytes [17] and chondrocytes [18] as well as the regeneration of cartilage defects in vivo [19]. Typically, most hydrogels are bioinert, so they need additional modification with proteins or cell-adhesive peptides to provide cell-adhesive properties [20–22]. In contrast, heparin is known to have specific affinities with various growth factors, extracellular matrix (ECM) proteins and cells [23]. Interestingly, some studies reported good cell-adhesive affinity of mesenchymal stem cells on heparin-modified surfaces [24,25].

Based on these previous reports, we hypothesized that heparin-based hydrogels could be a good culture matrix for human mesenchymal stem cell (hMSC) culture via direct interaction with heparin moieties. In this study, we sought to analyze the characteristics of heparin-based hydrogels for the culture of hMSCs. To see if serum proteins have an effect of heparin, cell culture on various surfaces was compared both in serum-containing and serum-free medium. Furthermore, it is known that the elasticity of matrix plays a significant role in regulating the fate of cells [26–30]. To address this issue, we prepared heparin-based hydrogel using a thiol-ene reaction in a wide range of elasticity ranging from 400 to 43,300 Pa after swelling. Also, to characterize the effect of heparin amount and gel elasticity separately, heparin-based hydrogels with various moduli but with a same amount of heparin were also compared.

2. Materials and methods

2.1. Materials

Heparin (a sodium salt from porcine intestinal mucosa, MW 12 kDa) was purchased from Cellsus Ins. (Cincinnati, IA, USA). PEG-DA (MW 3.4 and 6 kDa, 98% degree of substitution) and tetra-functional poly(ethylene glycol) sulfhydryl (PEG-SH4; MW 10 kDa) were purchased from SunBio Inc. (Anyang, Korea). 4-(2-Hydroxyethoxy) phenyl-(2-hydroxy-2-propyl) ketone (Irgacure 2959) was purchased from Ciba Specialty Chemicals Inc. (Basel, Switzerland). Dulbecco's modified Eagle's medium (DMEM), sodium pyruvate, nonessential amino acids, fetal bovine serum (FBS) and penicillin/streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). Human anti-integrin β1, β2, β3 or selectin antibody and goat IgG secondary antibody—H&L (Texas Red or fluorescein isothiocyanate (FITC)) were purchased from Abcam Inc. (Cambridge, MA, USA). The cell proliferation reagent WST-1 was purchased from Roche Ltd, (Basel, Switzerland). Formalin was purchased from Fisher (Pittsburgh, PA, USA). Anti-SSEA4 primary antibody was purchased from Santa Cruz Biotechnologies, Inc. (Santa Cruz, CA, USA). Mounting medium with 4′,6-diamidino-2-phenylindole (DAPI) was purchased from Vector Laboratories, Inc. (Burlingame, CA, USA). Phalloidin–FITC and Oil Red-O were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Preparation of heparin-based hydrogels

Heparin-based hydrogels were prepared by thiol-acrylate photopolymerization using Hep-SH and PEG-DA (Sunbio Inc. Anyang, Korea) [16]. For this, 40% thiolated heparin, prepared as reported previously [15], and 6 kDa PEG-DA were dissolved in phosphate-buffered saline (PBS) containing 0.1 w/v% photo-initiator, Irgacure 2959 in 70 vol.% ethanol, then exposed to 365 nm, 18 W cm^{-2} UV light using an OmniCure series 1000 light source (EXFO, Vanier, Quebec, Canada) for 10 s. By using th ethiol-ene reaction, heparin-based hydrogels were prepared by either changing the total concentration of precursors at the same molar ratio between the thiol group of Hep-SH and the acryl group of PEG-DA (labeledlabeled Hep-T-*x*, where *x* is the total polymer concentration) or by adjusting the amount of PEG-DA against a fixed amount of heparin (labeledlabeled Hep-*x*, where *x* is the total polymer concentration) (Table 1). For example, 10 wt.% heparin-based hydrogel was made by dissolving Hep-SH and PEG-DA (1:1 molar ratio of thiol group and acryl group; labeled as Hep-10) and 5.3 wt.% heparin-based hydrogel was made by dissolving Hep-SH and PEG-DA (1:0.3 molar ratio of thiol group and acryl group; labeled as Hep-5.3) in PBS for a fixed reaction condition (initiator concentration, light intensity and irradiation time). TCP, PEG hydrogel and agarose hydrogel were used as control groups. PEG hydrogels were prepared by using 10 kDa PEG-SH4 and 3.4 kDa PEG-DA (1:1 molar ratio of thiol group and acryl group) to have a similar network state [31], and thus similar mechanical properties, to those of the heparin-based hydrogels (labeled as PEG-x, which have similar mechanical properties to the corresponding Hep-*x* (Table 1)). Agarose hydrogels were prepared by using agarose type I (Sigma) at 1 wt.% agarose solution, which resulted in a hydrogel with a similar modulus to the optimized heparin-based hydrogel for hADSCs. Agarose powder was dissolved in PBS by heating the solution and then the gel was set by cooling at room temperature.

2.3. Characterization of heparin-based hydrogel

Moduli of various hydrogels were measured using a rheometer (Gemini, Malvern Instruments, UK, USA) equipped with a temperature controller at 37 °C and a solvent trap. Samples were analyzed by parallel plate geometry. An angular frequency of $\omega = 1$ rad s^{-1} and a strain of $\gamma = 0.1\%$ were selected to ensure the linear regime

Table 1
Elastic moduli, compositions and swelling ratio of various hydrogels.

Sample	Initial polymer conc. (wt%)	Abbreviation	Molar ratio (thio:acryl)	Elastic modulus before swelling (Pa)	Elastic modulus after swelling (Pa)	Swelling ratio	Initial amount of Hep(SH) (mg/ml)	Final amount of Hep(SH) (mg/ml)*
Heparin hydrogel	3	Hep-T-3	1:1	1,320	410	36.8 ± 12.7	10	8.3 ± 2.0
	5	Hep-T-5	1:1	4,260	1,680	25.2 ± 0.9	16.7	14 ± 2.0
	30	Hep-T-30	1:1	77,340	18,720	10.9 ± 0.9	100	94.9 ± 1.5
	5.3	Hep-5.3	1:0.3	1,000	400	43.6 ± 0.7	33.3	31.6 ± 1.6
	6.7	Hep-6.7	1:0.5	3,600	2,320	24.3 ± 0.1	33.3	31.9 ± 4.6
	10	Hep-10	1:1	12,650	6,290	15.9 ± 1.3	33.3	30.6 ± 2.9
	30	Hep-30	1:4	61,070	43,290	7.8 ± 0.4	33.3	30.5 ± 2.8
PEG hydrogel	5.3	PEG-5.3	1:0.3	970	390	44 ± 2.7	0	N.A.
	10	PEG-10	1:1	12,400	5,820	19 ± 0.3	0	N.A.
Agarose hydrogel	1	Agarose	N.A.	890	361	35 ± 0.7	0	N.A.

Hydrogels that were created by changing the total concentration of precursors at the same molar ratio between the thiol group and the acryl group were labeled Hep/PEG-T-*x* and those that were formed by adjusting the PEG-DA amount were labeled Hep/PEG-*x*, where *x* is the total polymer concentration. The heparin contents of the heparin-based hydrogels were measured by DMMB assay (mean ± standard deviation, $n = 4$).

* By DMMB asssay.