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Charge density is more important than charge polarity in enhancing osteoblast-like cell attachment on poly(ethylene glycol)-diacrylate hydrogel

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ABSTRACT

Two differently-charged monomers, namely, 2-(methacryloyloxy)ethyl-trimethylammonium chloride (MAETAC) and sodium methacrylate (SMA), were incorporated into poly(ethylene glycol)-diacrylate (PEGDA) hydrogels to investigate the effects of surface charge on the osteoblast-like cell attachment. The pKa of MAETAC and SMA was first determined, and then the physicochemical properties of the resultant polymers were characterized. Osteoblast-like MC3T3-E1 cells were also seeded on the hydrogels to evaluate the effect of charge polarity and charge density on the cell attachment. Results revealed that the pKa value of MAETAC and SMA was 9.4 and 4.8, respectively. Two charged monomers were incorporated into PEGDA. The zeta potential of hydrogels became positive or negative with the increasing concentration of MAETAC or SMA. Although different concentrations of charge monomer were incorporated into PEGDA hydrogel, no significant difference in surface morphology was found among each group. The swelling ratio and crosslinking density of modified hydrogels remained constant in phosphate-buffered saline. No difference was also observed on the contact angle between charged and non-charged hydrogels. However, more proteins were adsorbed on the charged hydrogels, irrespective of the charge polarity. The modification of hydrogels with charge monomer not only accelerated osteoblast-like cell attachment but also enhanced the expression of cell attachment-related genes. These findings indicated that charge density might be more important to affect the osteoblast-like cell attachment on the hydrogel in contrast to charge polarity. Through controlling the incorporation of charge monomer, designing the hydrogel and providing an opportunity to study the effect of charge on the cell behavior are possible.

1. Introduction

Bone tissue engineering combines scaffold, cells, and growth factors to create a mimicking native healthy bone biochemically and structurally [1]. The ideal scaffold is not passively tolerated by the osteoblast but actively promotes osteogenesis in a controllable manner. The osteoblastic behavior depends strongly on the chemical and physical properties of biomaterial surface. Moreover, the influences were started by triggering the adsorption of specific extracellular matrix (ECM) proteins and associated growth factors at the cell–material interface [2]. Therefore, focusing on the cell–material interaction, which may help guide the scaffold design, is increasingly important.

Osteoblast attachment plays a vital role in regulating the osteoblast proliferation and in switching the osteogenic differentiation [3]. In osteoblast, the cell attachment depends strongly on the physicochemical properties of the biomaterial surface, such as wettability [4], electrical charge [5], roughness [6], and rigidity [7]. However, how charge polarity and density affect osteoblast attachment remains controversial. For example, Liu found that the adsorption and conformation of osteopontin on the positively charged gold surface were more favorable for osteoblast attachment than on the negatively charged gold surface [8]. However, the induction of negative charge on the surface of titanium alloy formed an activated zone that promoted osteoblast attachment [9]. Meanwhile, Pernodet used sulfonated polystyrene and found that fibronectin as a major adhesion-medicating ECM protein underwent a transition from monolayer to multilayer adsorption at high positive charge density surface [10]. Nevertheless, Lee found that fibronectin adsorption cannot be improved on the positively charged silicon wafer surface even if the charge density was increased [11]. These confused results might

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Table 1
Preparation of different hydrogels.

Group	MAETAC (mM)	SMA (mM)
HG0	0	0
HGP50	50	0
HGP100	100	0
HGP200	200	0
HGN50	0	50
HGN100	0	100
HGN200	0	200

result from different experimental materials, which exhibited different protein adsorption abilities. Therefore, understanding the effect of surface charge on the osteoblast attachment is possible through controlling the density and polarity of surface charge employed on the material inert for cell attachment.

Poly(ethylene glycol)-diacrylate (PEGDA) is an important synthetic hydrogel used for bioconjugation, drug delivery, and tissue engineering [12]. PEGDA may also be used as a "blank-slate" to study the cell behavior because it forms a hydrated surface layer [13]. In contrast to traditional biomaterials, the extreme hydrophilicity inhibits the adsorption of adhesion-mediated proteins on the PEGDA. This condition thus excludes the influence of material itself on the osteoblast as much as possible [14]. Besides, the active acrylate group of PEGDA can be modified with charge molecules. In our previous study, a positively charged small molecule, 2-(methacryloyloxy)ethyl-trimethylammonium chloride (MAETAC), has been incorporated into PEGDA backbone in a controllable manner [15]. Through changing the concentration of charged monomer, the charge density of PEGDA is precisely controlled. Moreover, the positive charge modification improves the cell attachment on the PEGDA hydrogel [15]. By similar method, sodium methacrylate (SMA) as a negatively charged small molecule is incorporated into PEGDA to produce negative charge. Therefore, to evaluate the effect of different charge polarities and densities on the osteoblast attachment would be possible. This phenomenon is seldom reported in the literature.

PEGDA hydrogel is modified with MAETAC and SMA in this study, subsequently. The aim of this study is to understand the effect of charge polarity and charge density of hydrogel on the osteoblastic attachment. The polymers are observed using scanning electron microscopy (SEM), analyzed through Fourier transform infrared (FTIR) spectroscopy, and characterized by assessing the pKa, swelling ratio, zeta potential, contact angle, crosslinking density, and protein adsorption. At the same time, osteoblast-like MC3T3-E1 cells are seeded on the hydrogels to evaluate the effect of surface charge on the cell attachment.

2. Materials and methods

2.1. Material preparation

2.1.1. PEGDA synthesis

PEGDA was synthesized according to our previous study [15]. Briefly, 8 g of PEG (molecular weight 4000, Sinopharm Co. Ltd., Shanghai, China) and 0.25 g of triethylamine were dissolved in 36 mL of anhydrous dichloromethane. Subsequently, 0.43 g of acryloyl chloride was added dropwise into the mixture by a constant pressure funnel. The mixture was stirred overnight at room temperature. The resultant solution was washed with 2 M K_2CO_3 to remove HCl. Then, the mixture was stood at room temperature for 24 h and separated into aqueous and dichloromethane phases. The dichloromethane phase was dried with anhydrous $MgSO_4$. The polymer was precipitated in diethyl ether, filtered, and dried under vacuum at room temperature overnight. PEGDA was dissolved in DMSO-d^6 and analyzed by proton nuclear magnetic resonance (Avance 400 MHz; Bruker, Germany) to determine the degree of acrylation.

2.1.2. Hydrogel preparation

Hydrogels were prepared by dissolving PEGDA macromolecule at a final concentration of 20 wt% in 20 mM HEPES buffer saline (pH 7.4) containing 0.2% (w/v) Irgacure 2959 (Sigma-Aldrich, St. Louis, MO, USA). MAETAC (Sigma-Aldrich, St. Louis, MO, USA) and SMA (Sigma-Aldrich, St. Louis, MO, USA) were added into the hydrogel solution, subsequently (Table 1). The mixture was pipetted between glass slides with a 1 mm spacer and crosslinked by UV light (365 nm) at an intensity of 8 mW/cm^2 for 10 min.

2.2. Physicochemical characterization

2.2.1. SEM observation and FTIR analysis

The hydrogel samples were mounted on metal stubs and sputter coated with gold/palladium for 30 s after being lyophilized for 24 h. The surface of hydrogels was observed with SEM (JMS-6460, JEOL Ltd., Tokyo, Japan). Meanwhile, the structure of the dried hydrogels was characterized with FTIR spectrophotometer (Nicolet 5700, USA). The microscope used an ATR slide on a germanium crystal. The spectra were collected at a resolution of 4 cm^{-1} for 128 scans at an area of 150 μm × 150 μm.

2.2.2. Acid–base titration

MAETAC was prepared using deionized water at pH 4 at a concentration of 10 mg/mL. Then, 0.01 M NaOH solution was added in 10 μL increments. After every addition, the solution was stirred for 5 min. Then, the pH value was measured by a pH meter (PB-21, Sartorius, Germany). The pKa of MAETAC was defined as the pH value when half amount of quaternary ammonium was ionized. Similar method was used to determine the pKa value of SMA by adding 0.01 M HCl aqueous solution into SMA solution at pH 10. The pKa of SMA was defined as the pH value when half amount of carboxyl groups was deionized.

2.2.3. Swelling ratio, crosslinking density, and zeta potential

The crosslinked hydrogels of each group ($N = 6$) were immersed in phosphate-buffered saline (PBS) until achieving equilibrium swelling. The swollen hydrogel was blotted and weighed (Ws). The hydrogel was dried under vacuum for 24 h and weighted again (Wd). The swelling ratio was calculated as follows: Swelling ratio = (Ws − Wd) / Wd.

Crosslinking density (mol/mL) of hydrogels in physiological condition was calculated by the Flory–Rehner equation [16]: Crosslinking density $= -[\ln(1-v_2)+v_2+\chi_{12}v_2^2][V_1(v_1^{1/3}-2v_2/f)]^{-1}$. In this equation, χ_{12} represented the polymer solvent interaction parameter (for PEG-PBS system, it was 0.45); f is the crosslinking functionality. V_1 is the molar volume of PBS (18.062 mL/mol). v_2 is the volume fraction of hydrogel when it reached the equilibrium swelling state.

The swollen hydrogels of each group ($N = 4$) were ground into small particles and dried in a vacuum oven. Up to 5 mg dried particles of every group was weighed and diluted in 1 mL deionized water. The zeta potential was measured by a zetasizer instrument (Zen3600, Malvern Instruments, Malvern, UK).

Table 2
Primer sequences for RT-PCR.

Gene		Primer
FAK	Forward	CAGGCCTAGTGAAGGCTGTC
	Reverse	CAGCTGTCAGCATCTGCTTC
Intβ1	Forward	TGGCAACAATGAAGCTATCG
	Reverse	GTCCCACTTGGCATTCATTT
PI-3K	Forward	CCATGGAGCTGGAAGAGAAG
	Reverse	AGCTCCAATAGCGAGGATGA
TAL	Forward	CACAGCTCATTGCTGGCTAC
	Reverse	ATCTCCGTCAGGTTGGAAGA
GAPDH	Forward	AACGACCCCTTCATTGAC
	Reverse	TCCACGACATACTCAGCAC