riod. Using the above approaches, it was demonstrated that sustained delivery of two growth factors with different release modes induced far improved extent of *in vivo* angiogenic activity compared to a single growth factor delivery system.[10] Nevertheless, more controllable growth factor delivery systems are definitely needed for angiogenesis.

Previously, we reported the fabrication of macroporous biodegradable poly(lactic-*co*-glycolic acid) (PLGA) scaffolds based on a salt leaching/gas foaming method using a mixture of effervescent salts.[5,6] The resultant scaffolds had a highly porous and well interconnected pore structure without showing any surface skin layers. More recently, surface-modified PLGA scaffolds were prepared for enhancing adhesion and function of rat hepatocytes, bovine chondrocytes, and bone marrow stem cells by surface immobilization of bioactive molecules such as galactose, cell adhesive peptides, and hyaluronic acid.[15–18] These surface-modified scaffolds are expected to mimic the natural extracellular matrix (ECM), improving cell adhesion, proliferation, and differentiation. Among the ECM components, heparin has been known to specifically bind various angiogenic growth factors such VEGF, bFGF, and transforming growth factor-β.[19–21] Specific interactions occur between both 2-*O*-sulfate group and *N*-sulfate group of heparin, and lysine and arginine residues of the growth factors. Heparin in the ECM presumably plays a depot role for the growth factors, enabling them to diffuse out in a sustained manner. One of the heparin-binding growth factors, bFGF, is known to induce the proliferation of endothelial cells and has been studied as a potent mitogen for tissue regeneration, wound healing, and angiogenesis.[22] In addition, heparin bound bFGF improved recognition by cellular receptors and enhanced mitogenic activity. Furthermore, the binding of bFGF to heparin increased its stability against thermal denaturation and enzymatic digestion at physiological conditions. Based on the specific affinity between heparin and angiogenic growth factors, there have been numerous reports about controlled release of growth factors using heparin-conjugated natural polysaccharides such as alginate, chitosan, and hyaluronic acid.[8,9,23–25] These heparin modified hydrogel matrices loaded with growth factors were intended to be injected into a desired tissue defect site to induce local angiogenesis.

The objective of this study was to fabricate heparin-immobilized porous biodegradable scaffolds for local and sustained delivery of bFGF. Heparin was immobilized onto the surface of PLGA scaffolds by covalent conjugation between carboxylic groups of heparin and surface-exposed amine groups on the PLGA scaffolds. bFGF release profiles from heparin-immobilized PLGA scaffolds were comparatively examined and quantified. The bioactivity of the released bFGF fraction was measured by cultivating human umbilical vein endothelial cells (HUVECs) with the bFGF releasing scaffolds. The bFGF releasing scaffolds were implanted into subcutaneous pockets of mice for evaluating the extent of blood vessel formation.

MATERIALS AND METHODS

Materials

Poly(D,L-lactide-*co*-glycolide, lactide:glycolide ratio 50:50, RG504H, M_w 45,000) (PLGA) were purchased from Boehringer Ingelheim (Ingelheim, Germany). RG504H has an uncapped free carboxylic acid group in their terminal end. Poly(ethylene glycol)-bis-amine (PEG-bis-amine), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxylsuccinimide (NHS), and dicyclohexylcarbodiimde (DCC) (Aldrich, Milwaukee, WI) were used without further purification. PLGA 75:25 (Medisorb®, Mw 120,000) was purchased from Alkermes (Cincinnati, OH). Recombinant human fibroblast growth factor-basic (bFGF) was obtained from PeproTech (Rocky Hill, NJ). Heparin (M_w 18,000) was obtained from Sigma (St. Louis, MO). All other chemicals were of analytical grade.

Preparation of aminated PLGA

To introduce a terminal amine group to PLGA, a carboxylic acid terminal group of uncapped PLGA was activated by using DCC and NHS and reacted with PEG-bis-amine to prepare PLGA–PEG-NH_2 as previously described.[15,17] Five grams (0.11 mmol) of uncapped hydrophilic PLGA 50:50 (RG504H) was dissolved in 50 mL of methylene chloride. DCC (45.4 mg, 0.22 mmol) and NHS (25.3 mg, 0.22 mmol) were added into the polymer solution under magnetic stirring. Activation of the carboxylic acid end group in PLGA was accomplished for 12 h at room temperature. Insoluble dicyclohexylurea was removed by filtration, and the polymer with an activated carboxylic group was isolated by precipitation into anhydrous diethyl ether. The polymer precipitant was dried under vacuum. The coupling reaction of PEG-bis-amine to the activated PLGA was carried out by adding an excess amount of PEG-bis-amine. Five grams of the activated PLGA and 375 mg of PEG-bis-amine were reacted in 50 ml of anhydrous DMSO for 12 h at room temperature with stirring. The amine-terminated PLGA polymer (PLGA–PEG-NH_2) was precipitated by slowly dropping into cold ethanol. The collected gel mass was washed with an excess amount of ethanol and dried under vacuum.

Porous scaffold fabrication using PLGA blend

Aminated PLGA (PLGA–PEG-NH_2) was blended with PLGA 75:25 at a weight ratio of 10/90 to fabricate a macro-