INTRODUCTION

Heparin has been shown to have a high binding affinity for BMP-2, while also improving stability and bioactivity. Current carriers for BMP-2 have limitations associated with protein stability and rapid diffusion from the site of treatment. Therefore, heparin has been identified as a potential co-delivery factor for BMP-2 in bone repair applications to improve protein stability and bioactivity. The goal of this study focused on identifying the effects of co-delivering BMP-2 and heparin either by incorporating heparin into the collagen matrix or delivering a pre-bound BMP-2/heparin complex in the collagen matrix.

METHODS

Heparin integration into collagen matrix: Type I rat tail collagen (1.4 mg/ml) was mixed with heparin (10 µg/ml) and sodium bicarbonate, then deposited onto porous polycaprolactone (PCL) scaffolds and allowed to gel. The collagen gels were lyophilized overnight. BMP-2 was labeled with FITC using an antibody labeling kit, and imaged on the collagen matrices using confocal laser scanning microscopy before and after washing.

In vitro mineralization of hMSCs on collagen/heparin matrices: Two million cells suspended in cell culture media were deposited on each scaffold (with or without 0.5 µg BMP-2) and allowed to attach to the scaffold for 1 hour. Constructs were cultured in media supplemented with 6 mM β-glycerophosphate, 50 µg/ml ascorbic acid 2-phosphate (without dexamethasone) and incubated on a rocker plate to enhance perfusion of nutrients into the center of the scaffold.

In vivo segmental defect model: All surgical procedures were approved by the Georgia Tech IACUC. PCL scaffolds containing collagen or collagen/heparin matrices with or without 3 µg of BMP-2 were implanted into bilateral critical segmental bone defects, as described previously. BMP-2 was also pre-incubated with heparin before adding to the collagen matrix for comparison. The groups were as follows: (1) collagen, (2) collagen/heparin, (3) collagen + BMP-2, (4) collagen/heparin + BMP-2, and (5) collagen + BMP-2/heparin. Radiographs were obtained 2 and 4 weeks post-operatively (n = 5 - 9).

RESULTS

Image analysis parameters were determined at various fluorescent signal threshold levels to minimize noise levels for the quantification of FITC-labeled BMP-2 (Figure 2). A threshold of 50 was used for all future image analysis because the system noise levels were minimal (data not shown) and the measured fluorescent intensity area of 0 µg/ml FITC-BMP was reduced to minimal levels (Figure 2, highlighted by the red box). Analysis of FITC-BMP adsorbed onto collagen and collagen/heparin matrices showed that BMP-2 was retained in the matrix after a five-minute wash on a shaker plate when heparin was incorporated in the collagen matrix, but BMP-2 was not retained in the collagen matrix without heparin (Figure 3).

Micro-CT analysis of hMSCs on matrices indicated that BMP-2 delivered on collagen/heparin matrix induced significantly higher levels of mineralization (Figure 4). Neither did the collagen matrix with BMP-2 nor the collagen/heparin matrix without BMP-2 induce significant mineralization. A group of acellular collagen/heparin scaffolds was included to confirm that the observed mineralization was due to cell-mediated matrix formation.

DISCUSSION

In this study we examine the application of a heparinized collagen matrix to deliver BMP-2. Due to the high binding affinity of BMP-2 and heparin, the collagen/heparin matrix in the present study demonstrated improved in vitro mineralization of hMSCs. Preliminary in vivo results indicate that BMP-2 delivered in a collagen/heparin matrix or conjugated to heparin prior to adsorption on the collagen matrix is able to induce bridging of the defect within 4 weeks. This effect may be due to the affinity of BMP-2 to heparin as well as the stabilizing effects heparin has on the BMP-2 structure. Further quantitative analysis of the efficacy of each treatment will include micro-CT analysis at 4, 8, and 12 weeks, as well as assessing functional tissue integration through mechanical testing after 12 weeks. The results presented here suggest that the co-delivery of BMP-2 and heparin in a collagen delivery system may be beneficial for bone regeneration.

REFERENCES


ACKNOWLEDGEMENTS

This work was supported by the NIH Biotechnology Training Grant (T32-GM008433) and the Georgia Tech/Emory Center for the Engineering of Living Tissues (GTEC) NSF Grant EEC-9731643.