Introduction

PDGF is a protein growth factor released by platelets within the first 24 hours following tissue injury. PDGF synergizes with vascular endothelial growth factor to promote neovascularization, stimulates chemotaxis and proliferation of mesenchymal stem cells, osteoblasts, chondrocytes, fibroblasts and vascular smooth muscle cells, and accelerates fracture repair (Hollinger et al., 2008). Consequently, PDGF provides compelling therapeutic opportunities in orthopedic wound healing.

The therapeutic application of any growth promoting factor is dependent on its release at the proper time, duration, and dose with sufficient biological activity during the wound healing cascade. We previously developed two products, GEM 21S (Luitpold) and Augment™ Bone Graft (formerly GEM OS I), that consist of a combination of recombinant human PDGF-BB (rhPDGF-BB) with β-TCP, a synthetic bioresorbable bone matrix. GEM 21S is approved by the FDA for the treatment of bone loss associated with advanced periodontal disease, and Augment™ Bone Graft is in pivotal clinical trials for treatment of foot and ankle fusions. However, products that utilize rhPDGF-BB to enhance the osteoconductive and osteoinductive properties of bone allograft may have additional utility and appeal due to handling characteristics and physician preference.

The purpose of this study was to evaluate the in vitro and in vivo release profiles of rhPDGF-BB from the freeze-dried bone allograft (FDBA) cortico-cancellous chips, and to evaluate the biological potency and biochemical stability of the released protein.

Materials and Methods

The in vitro portion of the study was conducted using 0.3 mg/ml rhPDGF-BB in 20 mM sodium acetate, pH 6.0 that was combined with either Augment™Bone Graft, GEM 21S® β-TCP, or FDBA at a 1:1 (v/v) ratio in each case. The materials were allowed to hydrate with the rhPDGF-BB solution at room temperature for 10-15 minutes. A 0.3 mg/ml rhPDGF-BB solution was used as a control to monitor for binding of rhPDGF-BB to container surfaces. Following hydration, triplicate samples of the mixed material were combined with an elution buffer consisting of MEM tissue culture growth medium supplemented with 2% fetal bovine serum (FBS) at 37°C. The rhPDGF-BB recovered from the materials at 37°C was analyzed by ELISA, a cell-based alkaline phosphatase bioassay, SDS-PAGE, and high pressure size exclusion chromatography (HPSEC).

ELISA (R&D Systems, Quantikine) data for rhPDGF-BB released from test samples was normalized to the rhPDGF-BB control data at each time point tested. Cellular stimulation by rhPDGF-BB released from test samples was measured using an alkaline phosphatase-based bioassay with an MG-63 osteosarcoma cell line. The rhPDGF-BB protein stability was analyzed by HPSEC (TOSOH BioSep TSK-GEL HPLC column), and SDS-PAGE (BioRad 10-20% Tris-HCl gradient gels).

The in vivo release experiment was conducted at an AAALAC-approved facility with prior approval of the protocol by the facility’s IACUC review board. For the in vivo assessment, 125I-rhPDGF-BB was combined with Augment™Bone Graft, GEM 21S® β-TCP, or FDBA combined with β-TCP at a 4:1 v/v ratio, then surgically implanted into an 8 mm diameter calvarial defect in rats (n=4 per group). A radioactive measurement was taken immediately after implantation of the materials (time = zero) using a collimator-shielded Geiger counter. Measurements were then taken at 30 min, 1, 2, 4, 8, 24, 48, and 72 hr post-implantation. Radioactivity data at each time was normalized to the time zero measurement in each animal and plotted against time.

Results

When release kinetics were measured in the in vitro assay, rhPDGF-BB was released from GEM 21S® β-TCP in a rapid bolus manner with >90% released in the first hour, as shown in Figure 1. In contrast, the release of rhPDGF-BB from FDBA appeared to be delayed with less than 50% released after 24 hr of elution.

Conclusions

The study revealed that in vitro release of rhPDGF-BB from FDBA is delayed compared to the rapid release from Augment™Bone Graft and GEM 21S®. In contrast, rhPDGF-BB is rapidly released from FDBA/β-TCP in vivo with kinetics similar to both Augment™Bone Graft and GEM 21S®. Differences in release kinetics observed between in vitro and in vivo studies were attributed to differences in the two test environments with the in vivo system including contact with blood, proteases and other factors that are not present in the in vitro system. In all cases, the released protein was found to be biochemically stable and unaltered following contact with β-TCP or FDBA.

References