Introduction
Bone morphogenetic protein (BMP) has been successful in the treatment of challenging bone defects. However, its use is limited due to the high costs associated with treatment. The development of an alternative to BMP therapy using bioactive moieties could be a solution in treating osteoporotic bone and problematic bone defects.

We have developed a 3D electrospun scaffold composed of Poly(ε-caprolactone) (PCL) and Poly(vinyl phosphonic acid-co-acrylic acid) (PVPA) (collectively referred to as PCL/PVPA). The PCL fibre network mimics the extracellular matrix of bone and provides a substrate for cell attachment and proliferation. PVPA is able to chelate calcium ions due to the arrangement of the phosphorous atoms within the polymer. The PCL/PVPA scaffold can increase osteoblast attachment, proliferation and mineralisation in vitro.

Materials and Methods
A 10% w/v polymer solution of PCL (Mn = 80,000) in acetone was electrospun using an applied voltage 20kV, flow rate 0.05ml/min and a needle collector distance 15cm. Fibres were collected on a grounded collector plate to give a randomly orientated morphology. PCL scaffolds were functionalised with PVPA and formed into a 3D spherical structure (Figure 1B).

Human osteoblast cells (HOBs) (The European Collection of Cell Cultures, UK) (Cell line no. 406-05a) were cultured in Dulbecco’s modified Eagles medium (DMEM) (Gibco®, Paisley) supplemented with 10% FBS, antibiotics (100 U/ml penicillin, 100 mg/ml streptomycin) and 50µM ascorbic acid. HOBs were cultured on PCL/PVPA, PCL and tissue culture plastic (TCP) substrates for up to 28 days under standard conditions.

The attachment of osteoblast cells was assessed using Live/Dead staining, change in cell number was determined using the Hoescht DNA assay, and assessment of mineralisation was made using Alizarin red staining.

Results
After two hours, osteoblast cells had attached to all substrate types, however a greater number of dead cells were observed on PCL substrates when compared to PCL/PVPA substrates (Figure 1). A similar trend was observed after 24 hours.

There was an increase in osteoblast cell number on all substrate types over 21 days (Figure 2). At day 21, there were significantly (p<0.001) more cells on PCL/PVPA scaffolds when compared to PCL substrates.

There was a significant difference in mineralisation (quantification not shown) over 28 days (Figure 3). Mineralisation was greatest on PCL/PVPA scaffolds, less so on PCL scaffolds and even less so on tissue culture plastic substrates.

Discussion
Attachment of osteoblast cells was greater on PCL/PVPA scaffolds. The PCL/PVPA substrate is highly hydrophilic, in comparison, the PCL substrate is extremely hydrophobic. The PCL/PVPA hydrophilic surface is ideal for cell attachment and proliferation. The initial attachment of cells would also affect cell number. Overall there were significantly more cells on PCL/PVPA substrates; this is due to a greater number of cells initially attaching to the hydrophilic surface.

It is likely that the –COOH groups present in the PVPA polymer are able to chelate calcium ions and therefore produce calcium apatite which leads to an increase in alizarin red staining. The rate of mineralisation could also be increased due to the presence of phosphorous within the PVPA polymer, which would suggest that the critical concentration of phosphorous required to initiate mineralisation is reached more efficiently.

Significance
Active moieties such as PVPA can be used to functionalise biodegradable polymers in order to improve their biocompatibility.

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