INTRODUCTION: Bone is a regenerative tissue capable of osseous regeneration in fractures and contained defects due in part to its vascular network, reservoir of osteoprogenitor cells, and its constant turnover throughout life; however, its capability for orthotopic regeneration is limited. The cambium layer of the periosteum is a proven autologous source of cells for orthotopic bone generation, which are commended by their osteogenic potential, proliferative ability, and accessibility; however, the thinness of the cambium layer (2-5 cells thick) precludes its use in many applications.

Extracorporeal shock waves (ESWs) have been shown to cause rapid periosteal cambium cell proliferation and subsequent osteogenesis. This work investigates a novel strategy for orthotopic bone generation: applying ESW-therapy as a non-invasive, inexpensive, and rapid method for stimulating cambium cell proliferation, and combining these cells with a bioactive scaffold. A 1.25mJ/mm² ESW device (Ossatron, Alpharetta, GA) was chosen based on its effectiveness in a rat model; the treatment site (medial proximal tibia) was based on the rat model and because periosteum is routinely harvested from this site clinically.

Grps. 3 and 4 were used to compare ESWSP (Grp. 4) with CP (Grp. 3) when elevated and overlaid on a porous bioactive scaffold. At the time of surgery (4 days post-op), the medial tibial periosteum was scored with a scalpel proximal to the implant site, and a periosteal elevator used to elevate the periosteum off the bone. An anorganic bovine scaffold (1.5mm deep x 6.5mm; Geistlich, Switzerland) was implanted subperiosteally and the periosteum was sutured closed. At two weeks post-op, the limbs were fixed in formalin, imaged using micro-computed tomography (µCT), and then decalcified and prepared for histology. Tissue formed above, within, and to the sides of the scaffold, was demarcated as osseous, chondrocytic, osteoprogenitor (osteocyte, chondrocytic tissue), and non-osteoprogenitor tissue on H&E sections. Tissue within the pores of the scaffold was subdivided into upper half (bone from periosteum overlaid on the scaffold) and lower half (bone from residual progenitor cells left on bone surface). Periosteal callus away from the scaffold, which was undergoing endochondral ossification, served as an internal control for tissue appearance during histogenesis. The areas were recorded using ImageJ and all data are reported as the mean ± SEM of all the animals within each group. Student’s t-test was used to compare ESWSP groups with control groups; a p-value of <0.05 was considered significant.

RESULTS: Five of the six EWS groups had a marked thickening of the periosteum. There was a 4-fold, statistically significant, increase in the thickness of the EWS group when compared with the contralateral, non-stimulated controls (100±25µm vs. 25±2µm; p=0.007) and a 2.7-fold increase in EWSSP cell number (38±7 vs. 14±1; p=0.004).

On both µCT and histology, the normal and EWS groups demonstrated callus formation in response to the surgical elevation outside the scaffold boundary of both groups, which is consistent with prior reports. Only 1 of 6 samples in the CP + scaffold group demonstrated a thin layer of bone above the scaffold and the upper half of the scaffolds in this group were filled with non-osteoprogenitor/fibrous tissue (see Fig. 1B). All samples in the EWS group had osteoprogenitor tissue overlying the scaffold, which was infiltrating the scaffold pores. For both groups, there was bone infiltrating the lower scaffold pores coming from the cortical surface.

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