Evaluation of a Novel Regenerative Bone Adhesive in a Rabbit Critical Size Femoral Defect Model

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INTRODUCTION: Bone voids resulting from trauma, disease, or surgical intervention require effective biomaterials that support rapid healing and gradual integration into native bone. While calcium phosphate cements (CPCs) are commonly used, they lack adhesive properties and can persist in the body for years, delaying complete bone regeneration. A novel mineral-organic bone adhesive composed of tetracalcium phosphate and phosphoserine (TTCP-PS) has been developed to address these limitations with trace amounts of calcium carbonate added to the formulation for porosity and barium sulfate for radiopacity. TTCP-PS is injectable, load-bearing, osteoconductive, and designed to be gradually replaced by bone over several months. This study evaluates the in vivo biocompatibility, osteoconductivity, and resorption of TTCP-PS using a critical size distal femoral defect in New Zealand White rabbits over 26 weeks, with comparison to empty defect (sham) controls. The study was conducted in accordance with ISO 10993-6 and ISO 10993-12 standards for the biological evaluation of medical devices.

METHODS: 33 male and female skeletally mature New Zealand White rabbits were assigned to a 4-week (10 animals), 13-week (10 animals), or 26-week duration (13 animals), plus 1 baseline animal to be euthanized immediately following treatment. Under general anesthesia, a defect was made in the lateral condyle of the distal femur with a 2-3mm drill bit to serve as a pilot hole. The defect was then enlarged to create a final defect of approximately Ø6mm by 10mm depth. The defect site was implanted with the TTCP-PS. The procedure was repeated on the contralateral limb, but the defect was left empty as a sham control. The surgical sites were radiographed (lateral and anterior-posterior (AP) views) to evaluate defect locations and TTCP-PS placement. Animals were observed daily for general health, as well as the implantation sites for adverse reactions until healed. Upon euthanasia, a complete macroscopic observation of the tissue and viscera was conducted. Explant radiographs were taken for each femur and reviewed by an independent radiologist. Draining lymph nodes and any abnormal tissues were paraffin embedded and stained using hematoxylin and eosin (H&E). Implantation sites for both the sham and TTCP-PS were plastic embedded and stained using both Stevenel's Blue and H&E for a comprehensive evaluation of new bone formation and biomaterial degradation. The histomorphometric evaluation was performed using ImagePro® Plus 7 software and marked by the pathologist for measurements of new bone growth and residual implant material over time.

RESULTS: There were no clinical observations that indicated local and systemic toxicity caused by the test or control articles. TTCP-PS treatment sites saw a TTCP-PS Categorization/Reactivity Grade of slight reaction due to TTCP-PS eliciting more macrophages and giant cells than the sham control treatment. This is expected, as implanted materials often elicit some degree of inflammation. The macrophages associated with TTCP-PS sites had morphologic features (abundant vacuolated to granular cytoplasm) compatible with M2 (anti-inflammatory) macrophages which are important in tissue repair and are associated with phagocytosis of bioabsorbable implant materials. These giant cells are therefore considered to be a functional response to the TTCP-PS implant rather than a pathological and inflammatory response due to their engagement in turnover of the TTCP-PS to new bone. Inflammation associated with sham treatment sites is limited to that elicited by the procedure and is typically minimal to absent. New bone formation in TTCP-PS treatment sites was equal to that of the sham site at 4-weeks and was greater than the sham sites at 13 and 26-weeks. At all time points the sham sites had marked adipose tissue ingrowth. Tissue ingrowth into TTCP-PS was minimal at 4-weeks and largely consisted of new bone growth. At 13 and 26-weeks there was mild (>25-50%) to moderate (>50-90%) tissue ingrowth into TTCP-PS that consisted of new bone, fat, and hemopoietic cells demonstrating significant remodeling through 26 weeks. Histomorphometry was performed to evaluate new bone formation by comparing the TTCP-PS treated sites to the sham sites and residual TTCP-PS within the region of interest (ROI).

DISCUSSION AND CONCLUSION: This study demonstrates that TTCP-PS is a biocompatible, osteoconductive bone adhesive that supports rapid bone regeneration and material resorption in a critical size defect model. Unlike conventional calcium phosphate cements (CPCs), which have shown minimal remodeling over extended periods (up to four years), TTCP-PS exhibited substantial degradation and replacement by new bone within 26 weeks. The inflammatory response observed was consistent with a normal wound-healing cascade and was predominantly associated with M2 macrophages, which facilitate implant resorption and tissue repair. Histological and histomorphometric analyses confirmed increased bone formation over time in TTCP-PS treated sites compared to sham controls, validating the efficacy of the adhesive in promoting osteogenesis within a challenging defect environment. These findings support the continued development of TTCP-PS for orthopedic indications where load-bearing, regenerative, and degradable bone void fillers are needed.

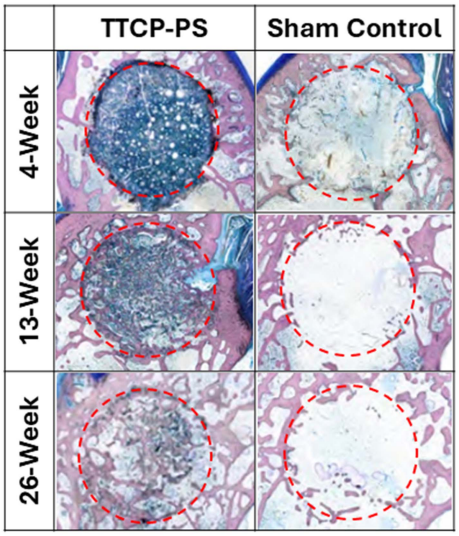

Table 1: Histomorphometry Results Reported as Percentage of the ROI and the group standard deviation (SD).

Time Point (Weeks)	Treatment Group	New Bone (% ROI)*	Residual TTCP-PS (% ROI)*
4 Weeks	TTCP-PS	5.6 ± 5.7%	72.0 ± 20.0%
	Sham	10.6 ± 9.4%	N/A
13 Weeks	TTCP-PS	18.9 ± 5.9%	24.1 ± 12.1%
	Sham	8.4 ± 9.0%	N/A
26 Weeks	TTCP-PS	19.8 ± 5.7%	17.5 ± 6.3%
	Sham	9.7 ± 7.9%	N/A

*Values are reported as mean ± standard deviation (SD). Residual implant material is not applicable (N/A) for sham groups.