Fetuin-Assisted Calcium Phosphate Hybridization Enhances Mineralization in Turkey Tendon for 3D Printed Titanium Implant Integration

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INTRODUCTION: Orthopedic arthroplasty for bone tumor, osteomyelitis, and prosthetic revision often requires wide-margin bone resection and extensive tissue dissection, leading to the loss of native tendon and ligament insertion sites [1]. Such procedures create bone defects and poor bone quality, compromising implant stability and integration [2]. Approximately 25% of prosthetic implants fail due to aseptic loosening, necessitating complex revisions with high morbidity [3]. Direct reattachment of tendons or ligaments to metal prostheses remains challenging because soft tissues lack the mineralizing capacity required for effective osseointegration [4]. The native enthesis provides a graded transition in composition and stiffness that minimizes stress concentration [5], its absence at the tendon–metal interface results in mechanical mismatch and loosening [6,7]. Calcium phosphate biomaterials promote osteogenesis but may cause uncontrolled mineral deposition and matrix degeneration [10–12]. Our previous studies showed that Fetuin-A, a natural inhibitor of ectopic calcification, forms calciprotein particles to regulate mineralization, enables controlled, graded mineral deposition on tendons [13,14,15,16]. In this study, we hypothesize that Fetuin-assisted calcium phosphate hybridization can improve tendon–metal integration evaluated on mechanical and biological attachment to 3D-printed titanium implants under our established turkey model.

METHODS: Bone marrow and flexor tendon tissues were harvested from 10-month-old heritage-breed turkeys (8–10 kg) for isolation of bone marrow–derived mesenchymal stem cells (BMSCs) and tendon cells. Cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C under 5% CO_2, with passages 3–5 used for experiments. Six coating conditions were prepared on 6-well plates: Control, CaP (calcium–phosphate), low- and high-concentration Fetuin (1 mg/ml and 5 mg/ml), and combined CaP + Fetuin groups (CaPLFetuin, CaPHFetuin). Solutions of $CaCl_2$ (100 mM + 30 mM L-histidine) and phosphate buffer (NaH_2PO_4/Na_2HPO_4, 15:85) were filtered and applied to form hybrid films, which were characterized by scanning electron microscopy. Turkey BMSCs and tendon cells were seeded (2×10^5 cells/ml) on the coated films and induced for osteogenic differentiation using medium containing β-glycerophosphate, ascorbic acid, and dexamethasone. Alkaline phosphatase (ALP) staining and qPCR were performed on day 7, and Alizarin Red S (ARS) staining at day 21. Relative gene expressions were quantified by RT-qPCR. Titanium alloy (Ti-6Al-4V ELI, Grade 23) plates with 76% porosity were fabricated by laser powder-bed fusion (EOS M290). Based on in vitro results, CaPHFetuin, CaP, and control were randomly assigned for in vivo testing in total 18 turkeys. After 12 weeks, specimens underwent pull-out mechanical testing (20 mm/min) and histological analysis with H&E, Masson's trichrome, and von Kossa staining to assess tendon–implant integration (Figure 1).

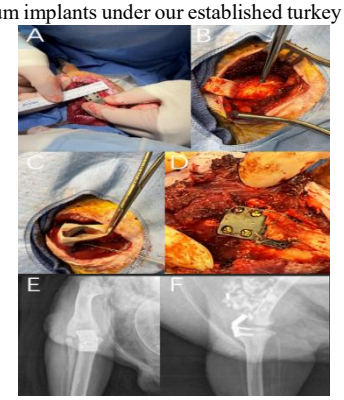

Figure 1: Tendon-plate turkey surgical procedure. A, 3D-printed titanium implant; B, mid-third patella tendon release; C, patella tendon sutured to titanium implant and soaked in solutions as planned; D, fixation the plate to proximal tibia; E, 3-month post-operative X ray AP view; F, 3-month post-operative X ray lateral view.

RESULTS: BMSCs exhibited enhanced osteogenic differentiation on calcium phosphate and Fetuin-coated films. ALP staining revealed markedly increased enzymatic activity in the CaP, LFetuin, HFetuin, CaPLFetuin, and CaPHFetuin groups compared to controls, with the CaPHFetuin formulation showing the strongest ALP induction. ARS staining confirmed enhanced calcium nodule formation in all treatment groups, with the CaPHFetuin film producing the largest and most abundant mineral deposits. These findings indicate synergistic effects of Fetuin and calcium phosphate on osteogenic differentiation of BMSCs. Similar trends were observed in turkey tendon-derived cells. ALP staining showed elevated osteogenic activity across Fetuin- and CaP-treated groups, while ARS staining demonstrated extensive mineral deposition, again most pronounced in the CaPHFetuin group. Gene expression analysis on day 7 further supported these findings: BMSCs cultured on CaPHFetuin films displayed significantly increased Osteocalcin (OCN) and Integrin-binding sialoprotein (IBSP) expression (**P < 0.001). In tendon cells, CaPHFetuin induced marked upregulation of RUNX2, OCN, and IBSP (**P < 0.001), indicating robust osteogenic commitment in both cell populations (Figure 2). In vivo mechanical testing showed higher peak pull-out strength in CaPHFetuin group (122.37 ± 8.55 N) compared with controls (113.26 ± 4.16 N). Histological analysis revealed extensive fibrous tissue ingrowth and organized collagen alignment at the tendon–implant interface, with clear evidence of graded mineral deposition within the inner tendon layers (Figure 3).

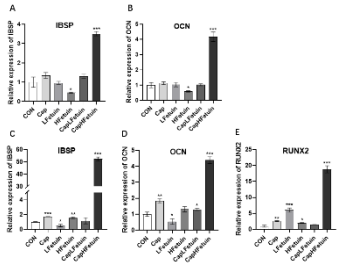

Figure 2: Analysis of qPCR of MBSC. A, the expression of IBSP; B, the expression of OCN;Analysis of qPCR of turkey tendon cell. C, the expression of IBSP; D, the expression of OCN; E, the expression of RUNX2. ∗P <0.05; ∗∗P <0.01; ∗∗∗P <0.001.

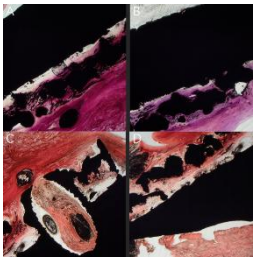

Figure 3: Images of hard tissue sections (200-300μm thick). A, H&E stain; B, Masson's trichrome stain; C and D, von Kossa stain.

DISCUSSION: Achieving robust soft tissue–metal integration is essential for the long-term success of orthopedic procedures involving tendon-to-bone repair. Fibrous ingrowth is mechanically inferior to the native enthesis, strategies that promote biomimetic mineralization of tendon tissue have emerged as promising solutions. Fetuin-A facilitates controlled calcium phosphate deposition by stabilizing nanocrystalline minerals, mimicking natural bone biomineralization. When combined with porous 3D-printed titanium structures, this biomimetic approach enhances mechanical interlocking and biological fixation. The turkey knee model provides a cost-effective, anatomically and biomechanically relevant platform for evaluating tendon-to-implant integration, offering greater translational relevance than small-animal models. This study demonstrated that CaPHFetuin enhanced osteogenic differentiation and histological findings implied the development of a neo-enthesis, a specialized, functionally graded interface resembling the natural tendon-to-bone attachment.

SIGNIFICANCE/CLINICAL RELEVANCE: This study presents a Fetuin-assisted calcium phosphate hybrid coating that promotes biomimetic mineralization and enthesis-like integration between tendon and titanium implants. The approach enhances osteogenic differentiation and tendon ingrowth, offering a promising strategy to improve soft tissue fixation and reduce implant loosening in complex orthopedic reconstructions.

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