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

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INTRODUCTION: Joint replacement surgeries for conditions such as bone tumors, osteomyelitis, and prosthetic revision often require extensive tissue removal resulting in the loss of natural insertion sites for tendons and ligaments around the joint [1,2]. To restore tendon/ligament function, surgeons sometimes need to reattach the tendon/ligament directly to metal prostheses, with the hope of achieving effective tendon-metal integration[3]. However, establishing a stable interface between tendon and metal is challenging, often resulting in tendon or ligament detachment. Detached tissues can adhere to surrounding soft tissues, leading to limited joint function. Titanium alloy, the primary material used in artificial joint prostheses, is bioinert, which hinders secure tendon attachment to the metal surface[4]. The significant difference in physical properties at the tendon-prosthesis interface also induces stress concentration, which increases the risk of fixation failure. Conventional methods rely on mechanical fixation rather than biological integration and are prone to long-term complications, including loosening and detachment, which affect patient mobility and functional outcomes. Enhancing the integration at the tendon-metal interface is essential for improving surgical efficacy and supporting functional rehabilitation. Our previous studies have shown that chemically treated tendons can promote tendon calcification, converting tendon tissue into bone-like tissue to support tendon-to-bone healing, as in ACL reconstruction[4]. This mechanism offers a promising foundation for tendon-to-metal implant integration. The goal of this study was to explore the potential for enhancing tendon-metal integration by developing a turkey model to test the effects of a calcium-phosphate film hybridized with Fetuin, a serum protein known as dual role in regulating mineralization and resembling bone tissue. It works by sustaining elevated calcium and phosphate levels in solution while selectively preventing apatite crystal growth outside collagen fibrils. [5].

METHODS: We examined the surface of the cell culture dish using scanning electron microscopy (SEM). As shown in Fig. 1, the coated surface exhibited significant structural alterations compared to the uncoated control group. Turkey tendon cells were collected from the tibias of 10-month-old heritage-breed turkeys (weighing 8–10 kg) with approval from the Institutional Animal Care and Use Committee (IACUC) and mixed with heparin sodium (NOVAPLUS1) in a syringe. Hybrid films of Fetuin and calcium phosphate were created on the surface of the culture plates (6-well plates), and six experimental groups were established based on film composition: (1) Control (ddH_2O), (2) Cap (calcium and phosphate solution), (3) LFetuin (1 mg/ml Fetuin), (4) HFetuin (5 mg/ml Fetuin), (5) CapLFetuin (calcium and phosphate solution combined with 1 mg/ml Fetuin), and (6) CapHFetuin (calcium and phosphate solution combined with 5 mg/ml Fetuin). Solutions for the LFetuin and HFetuin groups were prepared by dissolving Fetuin in ddH_2O at concentrations of 1 mg/ml and 5 mg/ml, respectively. Turkey BMSCs were seeded in the six-well plate coating with films and induced for osteogenic differentiation. After 7 days, ALP staining (Promega) was performed, and RNA was extracted and reverse-transcribed into cDNA (BIO-RAD) for qPCR analysis (Quantabio). After 21 days, ARS staining (Cyagen) was conducted. Based on the results from the in vitro testing, six patellar tendons were randomly assigned to two experimental groups, Group 1 (ddH_2O) and Group 2 (CapHFetuin) using a novel tendon–titanium turkey testing model.

RESULTS: ALP is an early marker protein associated with osteoblast differentiation. ALP staining demonstrated increased staining intensity in the Cap, LFetuin, HFetuin, CapLFetuin, and CapHFetuin groups compared to controls, indicating significantly elevated ALP activity. These findings suggest that both Cap and Fetuin enhance osteogenic differentiation in turkey tendon cells, with the CapHFetuin film markedly promoting ALP production. ARS staining was then performed to assess calcium nodule formation across films. Cells on Cap, HFetuin, CapLFetuin, and CapHFetuin films showed a greater propensity for calcium nodule formation, with CapHFetuin yielding the largest and most numerous deposits (Fig.2). On day 7 of differentiation, RNA was extracted for qPCR analysis. Expression of key genes in calcium deposition and bone mineralization, RUNX family transcription factor 2(RUNX2), Osteocalcin (OCN) and Integrin-binding sialoprotein (IBSP) were assessed, revealing significantly elevated RUNX2, OCN and IBSP expression (**$P < 0.001$) in cells on CapHFetuin films compared to controls (Fig.3). In biomechanical testing, compressive modules did not differ significantly between groups, and no significant differences in maximum failure strength or stiffness were observed in the suture pull-out test. Histological analysis demonstrated mineral deposition along the tendon inner layers with graded penetration and good tendon in-growth within our porous design titanium plate.

DISCUSSION AND CONCLUSIONS: Fetuin is generally known as a systemic inhibitor of ectopic calcification. It circulates in blood and binds calcium–phosphate nanocrystals, preventing them from aggregating and depositing in soft tissues. However, in our ex vivo culture model, the fetuin was bound within a CaP film on the surface. This localization might change its behavior, as it is not just floating in serum scavenging calcium–phosphate. In such collagen-rich substrate, the fetuin could serve as a controlled mineral source, slowly delivering ions where collagen fibrils are present. Instead of stopping all mineral deposition, Fetuin prevents uncontrolled precipitation in the medium, allowing targeted mineralization inside collagen fibrils, which is critical for bone-like tissue formation. Our results demonstrate that Calcium Phosphate–Hybridized films with Fetuin significantly enhance osteogenic differentiation in turkey tendon cells and showed mineral enabling deeper, controlled infiltration of minerals into the collagen network.

SIGNIFICANCE/CLINICAL RELEVANCE: These promising in vitro results mark an important initial step and provide proof of concept for future in vivo studies to determine whether pre-calcified tendons, which can be prepared in just 30 minutes[6], can improve the osteogenic performance of metal implants, thereby promoting ossification of tendons and ligaments attached to implants.

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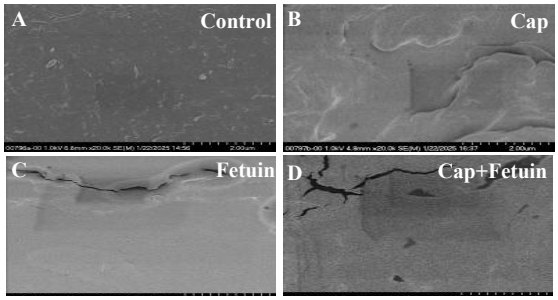

Fig.1 SEM micrographs of the films.
A, The surface of the control group; B, CaP film prepared in the absence of Fetuin; C, Fetuin film prepared in the absence of Cap; D, Hybrid films of Cap and Fetuin (Bar: 2.0 μm).

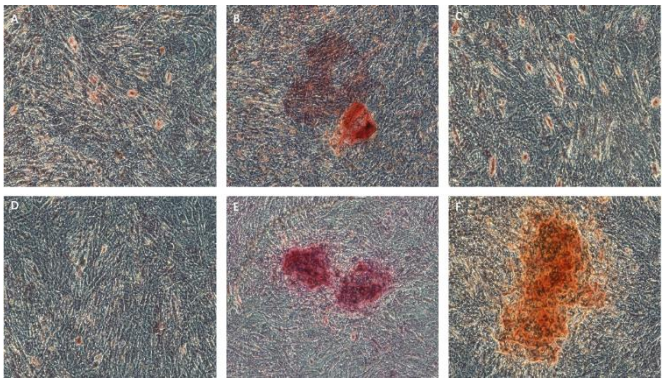

Fig.2 ARS staining.
A, Control; B, Cap; C, LFetuin; D, HFetuin; E, CapLFetuin; F, CapHFetuin.
Scale bars:100um.

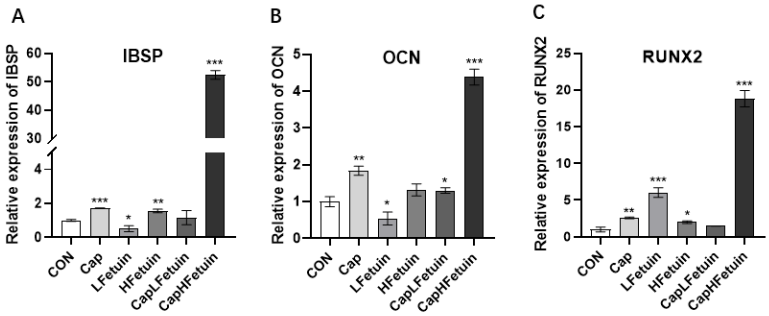

Fig.3 Analysis of qPCR.
A, the expression of IBSP; B, the expression of OCN; C, the expression of RUNX2.
∗P <0.05; ∗∗P <0.01; ∗∗∗P <0.001.