Supplemental Materials and Methods

Histochemistry and immunohistochemistry

TMJs were decalcified, embedded in paraffin, and cut sagittally into 5μm-thick sections. The most central sagittal sections of each joint were selected for safranin O (Sigma-Aldrich, St. Louis, USA, S2255) and immunohistochemical staining with antibodies for MGP (Santa Cruz, Dallas, USA, sc-66965), type II (Col-II, Santa Cruz, sc-52658) and type X (Col-X, Abcam, Cambridge, UK, ab58632) collagen, peroxidase-conjugated goat anti-rabbit IgG (Zhongshan Jinqiao Biotech, Beijing, China, SP-9001), and DAB substrate (Cowin Biotech, Beijing, China, CW0125). For negative controls, non-immune goat serum was substituted for the primary antibody.

RNA extraction and real-time PCR

Total RNA was extracted from condylar cartilage using the RNeasy Fibrous Tissue Midi Kit (QIAGEN, Hilden, Germany) and purified by the RNeasy mini kit (QIAGEN, Valencia, CA, USA), reverse-transcribed into cDNA, and amplified by a Applied Biosystems 7500 thermocycler using custom-made primers (**Supplemental Table.1**). Each sample was analyzed for three times and the mean values were calculated. The amount of target cDNA, relative to GAPDH, was calculated using the formula $2^{-\Delta\Delta Ct}$. The results were presented as the relative quantification of the target gene normalized to the 2-week control group.

Synthesis of BCP and CPPD crystals

BCP was synthesized as described previously [1]. Briefly, 50 ml 0.2 M NaPi (pH 6.7) was added to 42 ml 0.2 M $CaCl_2$ and stirred for 1 h at room temperature. The resulting crystals were collected by centrifugation at 3000 rpm, washed with H_2O, resuspended in 6.7 ml H_2O, and added to 67ml of 0.5 M HCl. The pH of the mixture was adjusted to between 8.5 and 10 and stirred overnight. The BCP crystals were then washed with H_2O, sterilized with 70% ethanol and freeze dried.

Polymorphs of CPPD were synthesized using methods described by Cheng and Pritzer [2]. Briefly, a liter of mixture containing 1 mM CaCl2, 0.8 mM MgCl2, 0.34 mM NaPPi, and 140 mM NaCl (pH 7.4) was prepared and shaken at 37^{o}C for 3weeks. The precipitates were collected by centrifugation at 3000 rpm, washed with H_2O, sterilized in 70% ethanol and freeze dried.

References

1. McCarthy GM, Cheung HS, Abel SM, Ryan LM. Basic calcium phosphate crystal-induced collagenase production: role of intracellular crystal dissolution. Osteoarthritis Cartilage 1998;6:205-13.

2. Cheng PT, Pritzker KP. The effect of calcium and magnesium ions on calcium pyrophosphate crystal formation in aqueous solutions. J Rheumatol 1981;8:772-82.