Introduction: Activated platelets are known to release many kinds of growth factors such as Platelet Derived Growth Factor (PDGF) and Transforming Growth Factor-β (TGF-β). Platelet-rich plasma (PRP) is a fraction of plasma in which platelets are concentrated and is reported to be utilized as a source of multiple growth factors that promote tissue repair. Recent basic studies have shown that allogenic PRP has a promotive effect on chondrocyte metabolism (1) (2). For the clinical application of PRP to cartilage defects, it is essential to use autologous prepared PRP in each case. However, no studies have clarified the effect of autologous PRP on human articular chondrocytes. In addition, it is unknown if autologous PRP has sufficient biological effect even in elderly patients with osteoarthritis. The purpose of this study was to examine the effect of autologous PRP on human chondrocytes obtained from elderly patients with osteoarthritis.

Materials and Methods: Plasma and cell preparation: Fresh blood (54ml) and cartilage tissue were obtained from five patients (mean age: 70y.o.) underwent total knee arthroplasty with their consent. Platelet poor plasma (PPP) and PRP were prepared using a platelet concentration system (SymphonyTM, DePuy). Chondrocytes were isolated with enzymatic digestion and cultured in monolayer. Isolated PPP and PRP were clotted with thrombin, followed by centrifugation and separation into fibrin gel and soluble supernatant. The soluble fraction was used for this experiment. The concentrations of PDGF and TGF-β1 in the fraction were measured with ELISA system (R&D Systems).

Cell culture: Isolated chondrocytes were cultured in DMEM/F12 containing 20%FBS at 37 degree and 5%CO2 air atmosphere, and the second passage cells were used for the following experiments. After 2 day pre-culture, the cells were cultured in serum-free medium for 6 hours and then cultured for another 48 hours under the following conditions; in the absence of serum (serum free group), in the presence of 10% Fetal Bovine Serum, 10% platelet poor plasma and 10% platelet poor plasma releasates (FBS, PPP and PRP groups respectively).

Cell proliferation and gene expression assay: WST-8 assay (cell counting kit-8) was used for cell proliferation assay. To analyze gene expression of types I, II collagen and aggrecan, relative quantitative real time RT-PCR assay was performed (TaqMan Gene Expression Assays, Applied Biosystems). Total RNA was isolated using TRizol reagent (Invitrogen) and then cDNA was made by reverse transcription of the isolated RNA. Amplification of the target gene was carried out by real time PCR system (Applied Biosystems 7500).

Results: The concentrations of PDGF and TGF-β1 in the PPP releasate were much higher than those in the PPP releasate. A direct sequential observation under microscope revealed that the PRP group has shown higher cell density than the other groups (Fig. 1A). Similarly, the WST-8 assay on day 2 showed significantly higher absorbance in the PRP group compared with other groups (Fig. 1B), which reflect an enhancement of cell proliferation. PRP significantly enhanced type II collagen synthesis by human chondrocytes, while their aggrecan synthesis was inhibited (Fig. 2).

Discussion: Articular cartilage has a poor intrinsic capability for self-repair. To accelerate regeneration of articular cartilage, the administration of growth factors is necessary, especially in elderly patients with osteoarthritis. Since PRP contains concentrated growth factors that are produced by platelets, the use of autologous PRP is thought to be safe and practical way for clinical application. PRP was shown to be useful in the field of bone formation, wound healing, periodontal tissue repair (3) (4) (5), and the promotive effects of allogenic PRP on chondrocyte metabolism have been also reported (1) (2). We demonstrated here for the first time that autologous PRP obtained from elderly patients with osteoarthritis stimulated proliferation and collagen synthesis of their chondrocytes. PRP is easy to prepare and utilize as both soluble platelet releasate and fibrin gel. The results of this study suggested the usefulness of autologous PRP for the treatment of cartilage lesion in osteoarthritis.