

Figure 5
Effects of rCCN2/CTGF on ALPase activity from MPL cells.

MPL cells. When MPL cells reached confluence, the culture medium was replaced with α -MEM containing 1% FBS, and the cells were then cultured with various concentrations of rCCN2/CTGF. After 48 hrs, the cell layers were collected, and ALPase activity was determined as described in "Materials and Methods." Values represent the averages \pm SD of 3 separate experiments. Asterisks denote statistically significant differences from the vehicle-treated control at the significance level of $*p < 0.01$.

in vitro [21]. As such, periostin itself may collaborate with CCN2/CTGF molecule in the remodeling of periodontal ligament.

As well as at the mRNA expression level, rCCN2/CTGF also stimulated collagen synthesis (Fig. 6). The turnover of collagen in the periodontal ligament is believed to be controlled by the balance between collagen synthesis and degradation. In this respect, up-regulation of collagen synthesis by rCCN2/CTGF may be accompanied by the dynamic control of gene expression of matrix metalloproteinases (MMPs) or tissue inhibitors of matrix metalloproteinases (TIMPs) for the remodeling of periodontal ligament [42]. It should be noted that induction of particular MMPs by CCN2/CTGF was reported to occur in vascular endothelial cells. Therefore, CCN2/CTGF may play a central role in promoting the remodeling of periodontal ligament through direct and indirect actions.

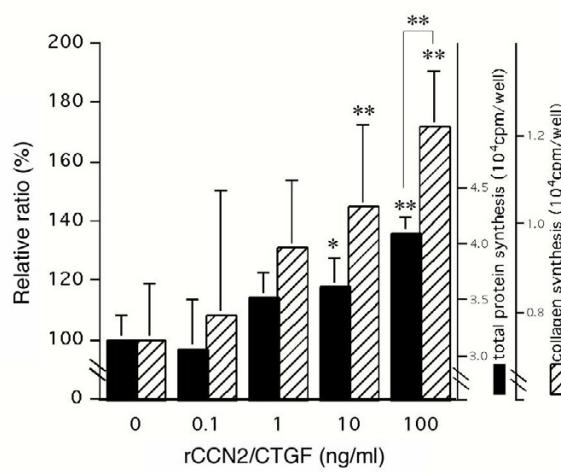


Figure 6
Effect of rCCN2/CTGF on the synthesis of collagen and total protein in MPL cells. Various concentrations of rCCN2/CTGF were added to confluent cultures of MPL cells, and the cells were cultured for 12 hrs. Then, the cells were labeled with [3 H] proline for another 12 hrs, after which their cell layers were collected for analysis. The radioactivities of [3 H] proline incorporated into total nascent proteins and collagenase-digestible portions were measured as described in "Materials and Methods." Slashed and closed boxes indicate the collagen and total protein synthesis, respectively. Values represent the mean average \pm SD ($n = 2$). Asterisks denote statistically significant differences ($^{**}p < 0.01$, $*p < 0.05$) from the vehicle-treated control, except the double asterisks specifying a significant difference between the stimulation levels of total protein and collagen synthesis (as indicated by the bracket). Data were computed with the results of 2 independent series of experiments with multiple sample numbers.

According to a previous report [25], CCN2/CTGF expression was observed in dental laminas, invaginating epithelium, and condensing mesenchyme at the bud stage. Afterwards, strong expression was observed in the enamel knot and preameloblasts; and CCN2/CTGF molecules were also detected in stratum intermedium and underlying dental mesenchyme, as well as in the dental epithelium. Additionally, during experimental tooth movement, CCN2/CTGF was highly expressed in osteoblasts around the periodontal ligament [42]. In this study, we confirmed the *ccn2/ctgf* expression and cell biological effects of CCN2/CTGF *in vitro*, by using MPL cell cultures. Overall, our results support the *in vivo* findings mentioned