Collagen-Phagocytosing Ability of Periodontal Osteoblasts at the Bone Surface*

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Summary. The collagen-phagocytosing activity of osteoblasts at the alveolar bone-ligament interface of rat mandibular first molars was investigated both histologically and histochemically. Alveolar bones of male Wistar rats (6 months old) were used in this study. Collagen-containing phagosomes appeared in cuboidal osteoblasts aligned on the bone surface. The 5.7% of the osteoblasts exhibiting alkaline phosphatase activity revealed collagen-containing phagosomes, and the collagen fibrils within the phagosomes were at various stages of degradation. In addition, acid phosphatase activity and the immunocytochemical distribution of cathepsin B were found in these collagen-containing phagosomes at similar locations. The presence of both enzymes in the phagosomes suggests that an intracellular degradation of collagen occurs. Therefore, in addition to the osteoblastic functions of synthesizing and secreting bone matrices, osteoblasts are also capable of phagocytosis and the intracellular disintegration of collagen. Our findings suggest that osteoblasts at the alveolar bone-periodontal ligament interface have a collagen-phagocytosing ability and play an important role in the physiological remodeling and metabolic breakdown of collagen fibrils of periodontal ligament without osteoclastic bone remodeling.

The high rate of remodeling and renewal of periodontal connective tissue (SODEK, 1976; SODEK and FERRIER, 1988) requires the orderly and specific removal of non-functional, denatured collagen fibrils followed by replacement with newly synthesized collagen (CARMICHAEL, 1982). There is a close correlation between the collagen turnover rate and cellular collagen phagocytotic activity *in vivo* (SVOBODA and DEPORTER, 1981; SVOBODA et al., 1981; EVERTS et al., 1996). Several studies have demonstrated the

phagocytosis and intracellular degradation of collagen fibrils by fibroblasts (TEN CATE, 1972; LISTGARTEN, 1973; GARANT, 1976; TEN CATE et al., 1976; BEERTSEN et al., 1978; MELCHER and CHAN, 1981; SCHELLENS et al., 1982) and cementoblasts (YAJIMA et al., 1989; SASAKI et al., 1990; YAJIMA and HIRAI, 1993) in the periodontium (see EVERTS et al., 1996 for review). Thus, fibroblasts and cementoblasts appear responsible for the secretion and resorption of collagen during remodeling and turnover.

The continuous remodeling of periodontal ligament fibrils is believed to occur at the bone-ligament interface during physiologic tooth eruption and migration (GARANT, 1976). The collagen-phagocytosing ability of osteoblasts in these processes is not yet clear. TAKAHASHI et al. (1986a, b) and EVERTS et al. (1994) reported that osteoblast-like bone-lining cells in mouse cultured-calvaria engulfed the non-mineralized collagen fibrils and degraded these fibrils intracellularly. Here, we focus on the collagen-phagocytosing function of osteoblasts at the alveolar bone-ligament interface of rat molars *in vivo*, describing our observations of osteoblasts involved in collagen-phagocytosis, and discussing possible roles of osteoblasts at this site.

MATERIALS AND METHODS

Fine structure

Six-month-old male rats of the Wistar strain were used. The animals were intracardially perfused with a solution of 2% paraformaldehyde-2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) under

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barbiturate anesthesia. Dissected mandibles were immersed in the same fixative for 12 h, and subsequently decalcified in cold 10% EDTA. The demineralized first molars and associated alveolar bone were further trimmed with razor blades to produce blocks of tissue, which were then postfixed with 1% OsO₄. These were stained *en bloc* with 1% aqueous uranyl acetate, dehydrated with ethanol and propylene oxide, and embedded in Epon 812. Ultrathin sections were stained with tannic acid, uranyl acetate and lead citrate, and examined with an electron microscope.

Enzyme cytochemistry

Demineralized tissue blocks were cut into thin buccal-lingual slices with a Microslicer (Dosaka EM, Kyoto, Japan). These slices were incubated for the demonstration of alkaline phosphatase (ALPase) (MAYAHARA et al., 1967; YOSHIKI et al., 1972) and acid phosphatase (ACPase) (NOVIKOFF, 1963) for 0.5–1 h at 37°C in the complete medium (containing substrate). Control tissues were incubated in the complete medium containing 2.5 mM levamisole (for ALPase), 10 mM NaF (for ACPase) or in a medium lacking the substrate. Following incubation, all specimens were postfixed in 1% OsO₄ and dehydrated before being embedded in Epon 812, as above. Ultrathin sections were stained with lead citrate only. No nonspecific enzymatic reaction products were observed in the control sections.

Immunocytochemistry

Animals were intracardially perfused with a solution of 4% paraformaldehyde–0.25% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3), under barbiturate anesthesia. Dissected mandibles were immersed in the same fixative for 12 h and subsequently decalcified in cold 10% EDTA. The demineralized specimens were embedded in Epon 812. Ultrathin sections were mounted on nickel grids. After etching with 1% sodium methoxide for 30 sec (WATANABE et al., 1988), the specimens were incubated with anti-cathepsin B (10 µg/ml) (Calbiochem, Cambridge, MA) at 4°C overnight. They were then treated with gold-labeled goat anti-rabbit IgG (gold particles 10 nm in diameter) (British BioCell International, Carrdiff, UK) at room tempera-

ture for 1 h. After the immunoreactions, the sections were stained with uranyl acetate and lead citrate. For immunocytochemical control experiments, sections were incubated with the non-immune rabbit serum, followed by the gold-labeled second antibody. No nonspecific deposits of gold particles were observed in the control sections.

Morphometric analysis

The incidence of osteoblasts possessing collagen-containing phagosomes was analyzed in the tissue blocks in which ALPase-activity was detected. Cell counts were made of 3 random sections in each of 12 blocks taken from 6 first-molars (in the right mandibles of 6 rats), directly under an electron microscope. The alveolar bone-periodontal ligament interface was divided into two zones, the cervical second and the apical second. Only ALPase positive-osteoblasts aligned along the bone surface were counted. The periosteum of the oral mucosal side in the corresponding portions of bone-ligament interfaces was used as a control. The percentage of osteoblasts with collagen-containing phagosomes was determined. Statistical analysis was carried out using the chi-square test and estimated confidence intervals.

RESULTS

In this study, observations were restricted to the alveolar bone-periodontal ligament interface of the buccal side from the mesial root of mandibular first molars.

At this side, osteoclasts were found only very occasionally, and most of the bone-lining cells were morphologically identified as osteoblasts (Fig. 1a); they showed cytoplasmic polarity and a well-developed rough endoplasmic reticulum, prominent Golgi apparatus, and numerous mitochondria. They also extended finger-like cell processes into the bone matrix and contacted the cell processes of osteocytes. Flattened pre-osteoblasts were often seen adjacent to cuboidal osteoblasts. Osteoblasts and pre-osteoblasts contacted each other by means of gap junctions (Fig. 2a). Large bundles of periodontal ligament fibrils located among the osteoblasts and pre-

Fig. 1 a. Osteoblasts (*Ob₁*, *Ob₂* and *Ob₃*) lie adjacent to the alveolar bone (*AB*) surface and between bundles of periodontal ligament fibrils (*BP*), and contain a well-developed Golgi apparatus and rough endoplasmic reticulum. They protrude their cell processes into the bone matrix. An osteoblast (*Ob₁*) contains an electron-dense collagen-containing vacuole (*arrowhead*). **b** and **c.** Enlargements of *Ob₁* shown in **a**. **c.** A different section in the same area of **b**. Collagen fibrils (*co*) within phagolysosomes (*arrowheads*) have partially lost their characteristic banding. The *insets* of **b** and **c** are higher magnifications of collagen-containing phagolysosomes. *Fb* fibroblast, *Oc* osteocyte, *Sf* Sharpey's fibers, *G* Golgi apparatus.

Fig. 1. Legend on the opposite page.

Fig. 2 a. An osteoblast (*Ob*) and pre-osteoblast (*pOb*) lie adjacent to the alveolar bone (*AB*) surface. They are in contact at gap junctions shown by *arrows*. **b-d**. Higher magnifications of the *framed areas* in **a**. **b** and **c**. Electron-lucent and electron-dense collagen-containing vacuoles. **d**. A cell process of an osteoblast is engulfing a collagen fibril (*co*). *BP* bundles of periodontal ligament fibrils, *Fb* fibroblast, *Sf* Sharpey's fibers.

osteoblasts penetrated the bone as Sharpey's fibers. At a short distance from the bone surface, elongated spindle or slender fibroblasts were oriented parallel to the direction of the collagen bundles. In contrast, cellular processes from these fibroblasts occasionally extended to the bone surface.

Certain osteoblasts included several collagen-containing vacuoles (Fig. 1b, c). Collagen fibrils within these vacuoles had partially lost their characteristic banding. Collagen fibrils were also often engulfed from one end by osteoblasts (Fig. 2a, d). Although some of the collagen fibrils surrounded by an electron-lucent material may not have been wholly internalized (Fig. 2b), this engulfing process is considered an early stage in phagocytosis (YAJIMA, 1986). The space between the membrane and fibrils became filled with electron-dense materials, and electron-dense collagen-containing vacuoles were observed in the osteoblasts (Fig. 2b, c). The collagen fibrils were digested within phagolysosomes, and exhibited various stages of degradation. In contrast, few of these fibroblasts were engaged in the phagocytosis of collagen fibrils. There was no evidence of collagen-phagocytosing activity in osteoclasts at the bone-ligament interface (data not shown).

Intense ALPase activity was observed on the external (periodontal ligament side) and lateral plasma membranes of all the osteoblasts with or without collagen-containing vacuoles, and on the plasma membranes of pre-osteoblasts at the bone-ligament interface (Fig. 3). In addition, there were reaction products over the extracellular matrix near the osteoblasts. However, the plasma membrane of the fibroblasts exhibited little or no enzyme activity in this experiment. ALPase activity was not seen in osteocytes.

In the osteoblasts, ACPase reaction products were seen in the trans-Golgi network, lysosomes and

phagolysosomes, including the collagen-containing vacuoles. ACPase activity was found within these collagen-containing phagolysosomes (Fig. 4). Heavy deposits of ACPase reaction products were localized in the electron-dense matrix found along the course of the fibrils within the phagolysosomes. Additionally, these products were often extensive in electron-dense varicosities, indicating the fusion of primary lysosomes with the phagosomes.

In the immunocytochemistry of osteoblasts, gold particles for cathepsin B were found in lysosomes and collagen-containing phagolysosomes (Fig. 5). Significant amounts of particles were detected in the electron-dense material within phagolysosomes.

The results of morphometric analyses are given in Table 1. Collagen-containing phagosomes at the cervical second and apical second were observed in 6.9% (95% confidence interval, $5.4\% \leq \mu \leq 8.4\%$) and 4.5% (95% confidence interval, $3.3\% \leq \mu \leq 5.7\%$), respectively, of the ALPase-activity positive-osteoblasts at the bone-ligament interface. The difference in these two zones was significant ($p < 0.05$). The average incidence of osteoblasts with collagen-containing phagosomes at the bone-ligament interface was 5.7% (95% confidence interval, $4.8\% \leq \mu \leq 6.7\%$). In contrast, at the periosteum of the oral mucosal side of the alveolar bone, collagen-containing phagosomes at the cervical second and apical second were observed in 0.19% and 0.22% of the ALPase-activity positive-osteoblasts, respectively, with no significant difference between these two zones. The average incidence of osteoblasts having collagen-containing phagosomes at the periosteum was 0.20%. The difference between the bone-ligament interface and the periosteum was significant ($p < 0.01$). Thus, osteoblasts at the bone-ligament interfaces were approximately 29 times more phagocytic for collagen compared with the osteoblasts of the periosteum.

Table 1. The incidence of osteoblasts with collagen-containing phagosomes at the bone-periodontal ligament interface and oral mucosal side periosteum of the rat alveolar bone.

	Bone-ligament interface		Periosteum (oral mucosal side)	
	cervical 1/2	apical 1/2	cervical 1/2	apical 1/2
No. of ALPase-positive osteoblasts	1145	1120	1056	906
No. of osteoblasts with collagen-containing phagosomes	79	50	2	2
Incidence (%)	6.9 ^a	4.5 ^b	0.19 ^c	0.22 ^d
Average incidence at the site (%)	5.7 ^e		0.20 ^f	

Significant difference between a and b ($p < 0.05$). No significant difference between c and d. Significant difference between e and f ($p < 0.01$).

Fig. 3. ALPase activity of osteoblasts and preosteoblasts. Heavy reaction products are observed along the periodontal-ligament side and lateral plasma membranes of osteoblasts (Ob_1 and Ob_2), and along the plasma membranes of a preosteoblast (pOb). An osteoblast (Ob_1) contains a collagen-containing vacuole (arrowhead). A fibroblast (Fb) exhibits very weak activity. The **inset** is a higher magnification of the collagen-containing vacuole within the osteoblast (Ob_1 , arrowhead). *AB* alveolar bone, *co* phagocytosed collagen fibril.

Figs. 4 and 5. Legends on the opposite page.

DISCUSSION

A rapid turnover of collagen in the rat periodontal ligament is essential for the continuous attachment of the roots to the alveolar bone, because rat teeth undergo physiologic drift, during which time the consequent bone formation and resorption occur spontaneously and continuously. The phagocytosis of collagen by fibroblasts is widely regarded to be an important pathway for the physiological degradation of collagen in the periodontium (SODEK and FERRIER, 1988; EVERTS et al., 1996). However, little attention has been paid to the mechanism and regulation of the remodeling of periodontal ligament fibrils at the alveolar bone surface.

Although no periosteum is formed at the alveolar bone-ligament interface, we were able to identify osteoblasts morphologically from periodontal ligament fibroblasts. In addition, we confirmed the presence of ALPase activity on the plasma membrane of osteoblast-like bone-lining cells. This enzyme is known to act as a differentiation marker in osteoblast differentiation (YOSHIKI et al., 1972; WLODARSKI and REDDI, 1986; GERSTENFELD et al., 1987). In practice, ALPase is used experimentally as a biochemical and cytochemical marker; osteoblasts are distinguished from fibroblasts by their ALPase activity (REDDI and HUGGINS, 1972). Thus, these cells, engaged in the phagocytotic process described above, were presumed to be osteoblasts due to their location, the ultrastructure of the cell organelles, and ALPase activity.

TAKAHASHI et al (1986a, b) demonstrated osteoblast-like cells at bone-forming sites, phagocytized collagen fibrils, and calcified bone matrix in cultured mouse calvaria. EVERTS et al. (1994) reported that osteoblast-like bone lining cells engulfed collagen fibrils in cultured mouse calvaria and that a relatively high percentage of the cells was involved in the engulfment of collagen.

The *in vivo* collagen-phagocytotic activity of osteoblasts has not been fully described. In this study, we observed the presence of intracellular collagen fibrils within osteoblasts at the alveolar bone-periodontal ligament interface *in vivo* and ACPase

activity in collagen-containing vacuoles. Our results show that these intracellular collagen fibrils are phagocytosed and are contained in phagosomes or phagolysosomes. A close relationship between ACPase activity and collagen degradation has been reported (WOESSNER, 1980). Previous studies have demonstrated that various lysosomal cysteine proteinases, such as cathepsins B and L, can degrade native type I collagen under acidic conditions (BURLEIGH et al., 1974; EVERTS et al., 1985; RIFKIN et al., 1991). These cathepsins were shown to play a crucial role in the digestion of phagocytosed collagen fibrils (EVERTS et al., 1985; VAN NOORDEN and EVERTS, 1991). Histochemical and immunohistochemical localizations of cathepsin B within the lysosomes of osteoblasts and osteocytes in rat bone have been reported (SANNES et al., 1986; OHSAWA et al., 1993). Our findings indicate that the immunocytochemical distribution of cathepsin B within collagen-containing phagolysosomes in osteoblasts is similar to the localization of ACPase. However, the activity and function of cathepsin B in collagen-containing phagolysosomes is still unknown, and further investigations are necessary to clarify the location and activity of these lysosomal collagenolytic enzymes within osteoblasts.

HEERSCHKE (1978) proposed that mononuclear fibroblast-like cells in close association with osteoclasts may participate in the removal of exposed non-mineralized collagen following osteoclastic demineralization. According to his hypothesis, these cells may phagocytose and subsequently digest the collagen intracellularly. It has been reported that fibroblasts in the vicinity of osteoclasts in the periodontal ligament contain many collagen-containing phagolysosomes (GARANT, 1976; BEERTSEN et al., 1978; RIFKIN and HEIJL, 1979). However, our finding that osteoblasts unrelated to osteoclasts at the bone-ligament interface directly phagocytose non-mineralized collagen fibrils is a phenomenon different from HEERSCHKE's hypothesis. We consider that these osteoblasts might be involved in the collagen remodeling of periodontal ligament during physiological tooth eruption and migration without bone remodeling.

In the present study, the osteoblasts at the alveolar

Fig. 4. ACPase activity in the collagen-containing phagolysosome within an osteoblast. Reaction products are present in the electron-dense matrix of a collagen-containing phagolysosome and lysosome (*ly*). *co* Phagocytosed collagen fibrils.

Fig. 5. Immunocytochemical staining for cathepsin B. Immunogold particles are deposited in a collagen-containing phagolysosome, particularly on dense material and a lysosome (*ly*). *co* Phagocytosed collagen fibrils, *M* mitochondrion.

bone-periodontal ligament interface were calculated by means of electron microscopic stereology, to have 29-times higher collagen-phagocytotic activity than that of the osteoblasts at the periosteum of the oral mucosal side. This result suggests that certain osteoblasts at the alveolar bone-periodontal ligament interface have definite collagen-phagocytosing ability, and are able to ingest and subsequently degrade collagen by means of their lysosomes. The osteoblasts are involved in the physiological remodeling and metabolic breakdown of collagen at this site. The collagen-phagocytotic activity was found to be significantly higher at the cervical second than the apical second. This difference may be due to differences in the remodeling activity of the sites. In contrast, osteoblasts at the periosteum showed a very low collagen-phagocytotic activity, suggesting a very low rate of collagen remodeling in this site under normal physiological conditions. Further investigations are necessary to clarify more precisely the activity of osteoblasts at both sites and whether all osteoblasts are capable of phagocytosis. It is also not clear whether the osteoblasts only function one way at a time, viz., bone matrix formation or collagen degradation.

We suggest that osteoblasts with collagen-containing phagosomes are responsible for the continuous remodeling of collagen fibrils at the bone-ligament interface, independent of osteoclastic activity. Collagen degradation during the turnover and remodeling of periodontal ligament tissue is probably achieved by fibroblasts and osteoblasts at the bone-ligament interface. A significant modulation of osteoblast function from primarily biosynthetic activities to degradative activities occurs in response to varying micro-environmental conditions at the bone-ligament interface.

In conclusion, we have shown that osteoblasts at the alveolar bone-periodontal ligament interface have collagen-phagocytosing ability and play a critical role in bone matrix degradation. Our findings suggest a new mechanism for the remodeling and metabolic breakdown of collagen at the bone-ligament interfaces.

REFERENCES

- BEERTSEN, W., M. BREKELMANS and V. EVERTS: The site of collagen resorption in the periodontal ligament of the rodent molar. *Anat. Rec.* 192: 305-318 (1978).
- BURLEIGH, M. C., A. J. BARRETT and G. S. LAZARUS: Cathepsin B₁. A lysosomal enzyme that degrades native collagen. *Biochem. J.* 137: 387-398 (1974).
- CARMICHAEL, D. J.: Biochemistry of the fibres of the periodontal ligament. In: (ed. by) B. K. B. BERKOVITZ, B. J. MOXHAM and H. N. NEWMAN: The periodontal ligament in health and disease. Persimmon Press, Oxford, 1982 (p. 73-102).
- EVERTS, V., W. BEERTSEN and W. TIGCHELAAR-GUTTER: The digestion of phagocytosed collagen is inhibited by the proteinase inhibitors leupeptin and E-64. *Collagen Rel. Res.* 5: 315-336 (1985).
- EVERTS, V., J.-M. DELAISSÉ, W. KORPER and W. BEERTSEN: Involvement of matrix metalloproteinases and cysteine proteinases in degradation of bone matrix. In: (ed. by) Z. DAVIDOVITCH: The biological mechanisms of tooth eruption, resorption and replacement by implants. EBSCO Media, Birmingham, 1994 (p. 85-97).
- EVERTS, V., E. VAN DER ZEE, L. GREEMERS and W. BEERTSEN: Phagocytosis and intracellular digestion of collagen, its role in turnover and remodelling. *Histochem. J.* 28: 229-245 (1996).
- GARANT, P. R.: Collagen resorption by fibroblasts. A theory of fibroblastic maintenance of the periodontal ligament. *J. Periodontol.* 47: 380-390 (1976).
- GERSTENFELD, L. C., S. D. CHIPMAN, J. GLOWACKI and J. B. LIAN: Expression of differentiated function by mineralizing cultures of chicken osteoblasts. *Devel. Biol.* 122: 49-60 (1987).
- HEERSCHE, J. N. M.: Mechanism of osteoclastic bone resorption: A new hypothesis. *Calcif. Tiss. Res.* 26: 81-84 (1978).
- LISTGARTEN, M. A.: Intracellular collagen fibrils in the periodontal ligament of the mouse, rat, hamster, guinea pig and rabbit. *J. Periodont. Res.* 8: 335-342 (1973).
- MAYAHARA, H., H. HIRANO, T. SAITO and K. OGAWA: The new lead citrate method for the ultracytochemical demonstration of activity of non-specific alkaline phosphatase (orthophosphoric monoester phosphohydrolase). *Histochemie* 11: 88-96 (1967).
- MELCHER, A. H. and J. CHAN: Phagocytosis and digestion of collagen by gingival fibroblasts *in vivo*: A study of serial sections. *J. Ultrastr. Res.* 77: 1-36 (1981).
- NOVIKOFF, A. B.: Lysosomes in the physiology and pathology of cells: contributions of staining methods. In: (ed. by) A. V. S. DE REUCK and M. P. CAMERON: Ciba Foundation Symposium on Lysosomes. Little Brown and Co., Boston, 1963 (p. 36-73).
- OHSAWA, Y., T. NITATORI, S. HIGUCHI, E. KOMINAMI and Y. UCHIYAMA: Lysosomal cysteine and aspartic proteinases, acid phosphatase, and an endogenous cysteine proteinase inhibitor, cystatin- β , in rat osteoclasts. *J. Histochem. Cytochem.* 41: 1075-1083 (1993).
- REDDI, A. H. and C. B. HUGGINS: Citrate and alkaline phosphatase during transformation of fibroblasts by the matrix and minerals of bone. *Proc. Soc. Exp. Biol. Med.* 140: 807-810 (1972).
- RIFKIN, B. R. and L. HEIJL: The occurrence of mononuclear cells at sites of osteoclastic bone resorption in experimental periodontitis. *J. Periodontol.* 50: 636-639 (1979).

- RIFKIN, B. R., A. T. VERNILLO, A. P. KLECKNER, J. M. AUSZMANN, L. R. ROSENBERG and M. ZIMMERMAN: Cathepsin B and L activities in isolated osteoclasts. *Biochem. Biophys. Res. Commun.* 179: 63-69 (1991).
- SANNES, P. L., B. H. SCHOFIELD and D. F. MC DONALD: Histochemical localization of cathepsin B, dipeptidyl peptidase I, and dipeptidyl peptidase II in rat bone. *J. Histochem. Cytochem.* 34: 983-988 (1986).
- SASAKI, T., T. SHIMIZU, C. WATANABE and Y. HIYOSHI: Cellular role in physiological root resorption of deciduous teeth in the cat. *J. Dent. Res.* 69: 67-74 (1990).
- SCHELLENS, J. P. M., V. EVERTS and W. BEERTSEN: Quantitative analysis of connective tissue resorption in the supra-alveolar region of the mouse incisor ligament. *J. Periodont. Res.* 17: 407-422 (1982).
- SODEK, J.: A new approach to assessing collagen turnover by using a microassay. A highly efficient and rapid turnover of collagen in rat periodontal tissues. *Biochem. J.* 160: 243-246 (1976).
- SODEK, J. and J. M. FERRIER: Collagen remodelling in rat periodontal tissues: Compensation for precursor reutilization confirms rapid turnover of collagen. *Collagen Rel. Res.* 1: 11-21 (1988).
- SVOBODA, E. L. A. and D. A. DEPORTER: Regional variation in collagen phagocytosis in rat gingiva: an electron microscope stereologic investigation. *J. Periodont. Res.* 16: 298-301 (1981).
- SVOBODA, E. L. A., A. SHIGA and D. A. DEPORTER: A stereologic analysis of collagen phagocytosis by fibroblasts in three soft connective tissues with differing rates of collagen turnover. *Anat. Rec.* 199: 473-480 (1981).
- TAKAHASHI, T., N. KURIHARA, K. TAKAHASHI and M. KUMEGAWA: An ultrastructural study of phagocytosis in bone by osteoblastic cells from fetal mouse calvaria *in vitro*. *Arch. Oral Biol.* 31: 703-706 (1986a).
- TAKAHASHI, T., S. SO, D. WANG, K. TAKAHASHI, N. KURIHARA and M. KUMEGAWA: Phagocytosis of different matrix components by different cell types at bone-forming sites in cultured mouse calvariae. *Cell Tiss. Res.* 245: 9-17 (1986b).
- TEN CATE, A. R.: Morphological studies of fibrocytes in connective tissue undergoing rapid remodelling. *J. Anat.* 112: 401-414 (1972).
- TEN CATE, A. R., D. A. DEPORTER and E. FREEMAN: The role of fibroblasts in the remodeling of periodontal ligament during physiologic tooth movement. *Amer. J. Orthodont.* 69: 155-168 (1976).
- VAN NOORDEN, C. J. F. and V. EVERTS: Selective inhibition of cysteine proteinases by Z-Phe-AlaCH₂F suppresses digestion of collagen by fibroblasts and osteoclasts. *Biochem. Biophys. Res. Commun.* 178: 178-184 (1991).
- WATANABE, M., T. WATANABE, Y. ISHII, H. MATSUBA, T. FUJITA, E. KOMINAMI, N. KATSUNUMA and Y. UCHIYAMA: Immunocytochemical localization of cathepsin B, H, and their endogenous inhibitor, cystatin β , in islet endocrine cells of rat pancreas. *J. Histochem. Cytochem.* 36: 783-791 (1988).
- WLODARSKI, K. H. and A. H. REDDI: Alkaline phosphatase as a marker of osteoinductive cells. *Calcif. Tiss. Int.* 39: 283-285 (1986).
- WOESSNER, J. F. JR.: Collagenase in uterine resorption. In: (ed. by) D. E. WOOLLEY and J. M. EVANSON: *Collagenase in normal and pathological connective tissues*. John Wiley and Sons, Chichester, 1980 (p. 223-239).
- YAJIMA, T.: Acid phosphatase activity and intracellular collagen degradation by fibroblasts *in vitro*. *Cell Tiss. Res.* 245: 253-260 (1986).
- YAJIMA, T. and T. HIRAI: Histochemical study on remodeling and degradation of collagen fibrils at the periodontal ligament-cementum interface. *Dent. Japan* 30: 5-13 (1993).
- YAJIMA, T., A. MATSUO and T. HIRAI: Collagen phagocytosis by cementoblasts at the periodontal ligament-cementum interface. *Arch. Histol. Cytol.* 52: 521-528 (1989).
- YOSHIKI, S., T. UMEDA and Y. KURAHASHI: An effective reactivation of alkaline phosphatase in hard tissues completely decalcified for light and electron microscopy. *Histochemie* 29: 296-304 (1972).

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