529 Effect of Local Administration of Estrogen on Experimental Tooth Movement in Ovariectomized rats. T. Yamashiro* and T. Takano-Yamamoto (Dept. of Orthodontics, Fac. of Dentistry, Osaka Univ. Osaka, Japan)

We have previously shown ovariectomy increased, and on the contrary, the replacement of estrogen decreased the rate of orthodontic tooth movement. The purpose of the present study was to investigate histomorphometrically the effect of local administration of estrogen on the remodeling of alveolar bone during experimental tooth movement in ovariectomized rats. Forty female Sprague Dawley rats, 6 weeks of age and weighing 150-160 g, were ovariectomized (OVX) or sham-operated and 14 days later the maxillary first molars were moved buccally with an appliance fabricated from nickel-titanium wire at a commencing force of 10 g for 18 days. In OVX rats, 20 μL of 10^{-9} M 17βE2 was injected locally into the submucosal palatal area of the root bifurcation of the right and left first molars every two days. Animals were sacrificed and maxillary alveolar bones were examined by histomorphometry at pressure and tension regions. 17βE2 caused the decrease in osteoclast number(N.Oc/BS), osteoclast surface(Oc.S/BS) and mineral appositional rate, which were elevated by ovariectomy, compared to those of sham operated rats at both of pressure and tension regions. At the pressure region, 17βE2 decreased osteoblast surface(Ob.S/BS), but caused no difference of that at the tension site. These findings suggest that the replacement of estrogen in the OVX rats decreases bone turnover elevated by ovariectomy in the rat alveolar bone. During experimental tooth movement, estrogen may have different effect on bone formation in the alveolar bone between pressure and tension region.
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530 Osteoblast Gene Regulation in Healing Tooth Sockets. M.LITZ*, K.NGUYEN, J.ROSSERT, B. DE CROMBRUGGHE and R.N.D'SOUZA (Univ. of Texas Houston, Health Science Center and M.D.Anderson Cancer Center, Houston, Texas, USA).

Like bone development, wound healing after tooth extraction follows an orderly series of predictable events where osteoblasts produce a type I collagen-enriched matrix. While the experimental model of healing after tooth extraction is well established, there is little information available on the molecular events leading to bone formation by osteoblasts. Mutational analyses of transgenic mice with α1(I) or α2(I) collagen promoter-reporter gene constructs have identified DNA segments that drive lineage-specific expression in osteoblasts during osteogenesis.Significantly, TGF-β1, a morphogen in the TGF-β gene superfamily, has been shown to interact with discrete cis-acting elements within these regions to stimulate type I collagen transcription. These studies were designed to test the hypothesis that these DNA segments are also utilized by osteoblasts regenerating bone after tooth extraction. Established transgenic mice harboring various regions of the α1(I) promoter linked to reporter genes that code for β-galactosidase and luciferase were used (a) to qualitatively and quantitatively assess whether DNA sequences that confer osteoblast-specific expression during embryogenesis are active in bone regeneration and, (b) to correlate reporter gene activity with that of endogenous α1(I) collagen and TGF-β1. After extraction of first maxillary molars in four week-old transgenic mice (positive and control littermates), serial sections were prepared through healing sockets at various postoperative intervals. X-gal histochemical staining and assays for luciferase activity were performed along with in situ hybridization using ^{35}S UTP-labeled riboprobes to compare transgene and endogenous gene expression respectively. Our results indicate that the 0.9 kb to 3.2 kb region of the α1(I) collagen promoter contains genetic elements that can drive reporter gene activity at two days after extraction and that transgene expression patterns correlated closely with the distribution of endogenous α1(I) collagen mRNA and TGF-β1. We conclude that the healing tooth socket is a valuable model to study osteoblast-specific activation mechanisms in bone regeneration. Studies were supported by NIH grants DE10517 (RD), BRSG-93 (RD), T35 DE07252 (KN) and HL41264 (B.d.C).

531 Effect of DBM on the Healing of Intramembranous Bone Graft. Y.M. DENG*, N. SAMMAN, and A.M. RABIE (Faculty of Dentistry, The University of Hong Kong, Hong Kong)

Bone formation occurs by either endochondral (EC) or intramembranous (IM) ossification. Previous work suggested that subcutaneous implants of demineralized EC and IM bone matrices both induced EC ossification With respect to the biochemical differences of both matrices, it is unclear why should they possess identical osteoinductive properties. The purpose of this study was to investigate the mechanism of healing of autogenous IM bone graft, in both the presence and in the absence of DBM, and to compare the healing of IM bone grafts to that of DBM alone. Twelve adult New Zealand White rabbits, two defects each were used as the experimental model. Critical-sized (10 x 5 mm), full thickness bony defects in the parietal bone, devoid of periosteum were filled with IM bone graft (mandible) alone, demineralized cortical bone matrix (DBM) alone, combined DBM-IM bone graft, and left unfilled. Histologic changes were examined 14 days later. The IM bone graft showed no cartilage intermediate stages and the healing proceeded through a route of entry for osteoblasts, the DBM implant was characterized by the presence of cartilage stage, and the DBM-IM bone graft showed cartilage intermediate stage In the latter two groups, the healing proceeded throughout the whole defect It is concluded that the IM bone graft alone heals directly through IM ossification, and in the presence of DBM, the healing is through EC ossification. This study was supported by the CRCG 372/251/6435, The University of Hong Kong, and IADR Norton M, Ross Fellowship, 1994.

532 Expression of Osteopontin mRNA in Bone Wound Healing. J. CHEN, H.F. THOMAS, H. JIN and D. M. RANLY* (Dept. of Pediatric Dentistry, The University of Texas Health Science Center, San Antonio, Texas, USA).

Osteopontin (bone sialoprotein II, OPN) is a major component of extracellular matrix proteins in bone, dentin and other mineralized connective tissues. Previous studies have suggested that OPN may be involved in formation, resorption and remodeling of bone. To investigate the OPN gene expression in an osseous wound healing model in vivo we created defects in rat femoral bone. Fifteen, 3-month-old rats were used in these experiments. Under general anesthesia, a defect, approximately 2 mm in diameter, was made in the mid-region of the lateral aspect of the femoral diaphysis using a dental bur. Animals were sacrificed at 6 and 8 days after surgery, respectively. Defined tissue samples encompassing the defect were carefully dissected and half of the tissues processed for histological examination and *in situ* hybridizaiton, and the other half quickly frozen in liquid nitrogen for isolation of total RNA. At day 6 after surgery, it was found that callus bone underneath the periosteum was deposited on the defect surface in a trabecular pattern and numerous delicate spicules of woven bone occupied most part of the wound. *In situ* hybridization with a [^{35}S]-labeled rat OPN cRNA probe revealed that the bone-forming cells expressed high level of OPN mRNA. The signals were concentrated in the plump osteoblastic cells lining on the newly formed bone tissue that was not fully mineralized. OPN signals were also seen in the cells in endosteal space in callus bone. Bone marrow cells and connective tissue cells between the bone trabecula showed no hybridization with the OPN probe. It was of interest to note that osteocytes located near the defect site showed much higher level of OPN signal compared with their counterparts in the intact sites of normal bone. It seemed that these osteocytes were re-activated by the creation of the wound defect or the subsequent repair process. At 8 days, the wound healing appeared to be more complete and the *in situ* hybridization signals were diminished, consistent with the results of Northern hybridization. These results indicate that the expression of OPN is important in the repair of bone as well as in normal bone remodeling.

533 Osteopontin and Bone Sialoprotein are Prominent Constituents of Sharpey's Fibers M.D. McKEE* and A. NANCI (Laboratory for Electron Microscopy, Department of Stomatology, Faculty of Dentistry, Université de Montréal, Québec, Canada)

Osteopontin (OPN) and bone sialoprotein (BSP), two major, non-collagenous, extracellular matrix proteins found in mineralized tissues such as bones and teeth, have been implicated in regulating calcification and mediating cell-and matrix-matrix adhesion. With regard to the latter function, we have investigated, using electron microscopy and immunocytochemistry, the structure and composition of Sharpey's fibers, *i.e.* the terminal extensions of periodontal ligament collagen fiber bundles that insert into alveolar bone at one end, and cementum at the other end. Together, these form a composite structure which provides the mechanical basis for tooth support within the alveolar socket. Wistar rats of various ages, and having molars at different stages of development, were fixed by vascular perfusion with aldehyde and the mandibles and maxillae were decalcified and embedded in LR White resin. Sections prepared for electron microscopy were processed for immunocytochemistry using polyclonal antibodies against either OPN or BSP (antibody LF-87), followed by incubation with protein A-gold complex. Immunolabeling patterns for both proteins over alveolar bone and cementum revealed an abundance of gold particles associated with periodontal ligament insertion sites corresponding to Sharpey's fibers. Immunoreactivity at these sites was associated with a network of finely-granular, organic material enveloping many individual collagen fibrils throughout Sharpey's fibers, and with more extensive and irregular patches of matrix surrounding the fibers At the bone surface, Sharpey's fibers frequently formed a 'fringe' of mineralized matrix, intensely labeled for both OPN and BSP, that was often separated from the underlying bone matrix proper by an OPN-rich cement line. In conclusion, these ultrastructural data identify OPN and BSP as major components of Sharpey's fibers. Furthermore, they suggest that intimate spatial associations and cohesive molecular interactions among collagen, non-collagenous proteins such as OPN and BSP, and mineral in Sharpey's fibers might collectively generate a matrix-mineral composite whose architectural and biomechanical properties are capable of withstanding the tensile forces of mastication. Supported by the MRC of Canada and the FRSQ of Québec.

534 Remodeling of Rat Alveolar Bone after Cessation of Bisphosphonate Treatment. Y. TAYA* and T. AOBA (Dept. of Pathol., The Nippon Dental Univ., Tokyo, JAPAN)

We previously reported that, when 1% HEBP (1-hydroxyethylidene-1,1-bisphosphonate) in drinking water was given continuously over several weeks, the periodontal ligament space of rat molars was occupied by osteoid, giving rise to ankylosis. In the present study, we aimed at investigating the fate of preoccupied osteoid and the reconstruction of periodontal ligament after cessation of the HEBP treatment. Fifteen Wistar rats (about 100 g b.w.) were kept with water containing 1% HEBP for the initial 28 days and thereafter HEBP-free water for various periods (4-28 days). In control groups, the animals were given either the water without HEBP from the beginning or the HEBP-containing water throughout the entire experimental periods. Techniques used entailed histochemistry, bone morphometry and bone labelings with tetracycline. Remarkable findings were that 1) once mineral deposition of the preoccupied osteoid occurred around 7 days after cessation of the HEBP administration, osteoclastic resorption became discernible; 2) at the same time fewer odontoclasts were also discerned around the ankylosis sites; and 3) ankylosis was remedied within 2 to 3 weeks under the HEBP-free regimen, followed by reconstruction of the periodontal ligament with new collagen formation. The overall results indicate that the osteoid mineralization and the subsequent activation of osteoclasts are required for recovery of the periodontal structure disturbed by HEBP.

535 The variation of Fluoride Levels with different methods of infusion of Tea. S ABAYARATNA, (Ministry of Health, Sri Lanka)

Tea, a beverage which is widely consumed in Sri Lanka, is infused by different methods depending on the Socio Economic status of the drinker. The F levels of the brew was measured after each method, using the Orion SA 270 pH ion meter.

1. The variation of fluoride levels after addition of milk was investigated using 76 tea samples. The reduction of F by almost 25% was highly significant.
2. Using a larger quantity of tea leaves for the infusion resulted in a stronger liquor which had significantly more F. The single strength brew had 1.63 ppm whilst the double strength had 3.6 ppm.
3. Some infused the tea leaves twice giving the second brew to the children. The F content of the second brew (0.36 ppm) was significantly less than that of the first (2.61 ppm).
4. Certain people infuse the tea for a longer time hoping to get a stronger brew. The fluoride levels analysed at 10, 15, 20, 25 & 30 minute intervals did not show a significant difference with time.
5. Sometimes the tea is brewed in the mornings and the tea leaves kept and re-used in the afternoon. More fluoride could be extracted by boiling the tea leaves. The increase in F levels from 7.2 ppm to 9.8 ppm after 2 minutes of boiling was highly significant.

The study showed that in a cup of tea the addition of milk and the use of the second infusion lowered the F content and that using a larger quantity of tea leaves and boiling the leaves produced a significant rise in Fluorides.

536 The Influence of Calcium Intake and Hardness of the Enamel. PUDJONIRMOLO* (Trisakti University, Jakarta - Indonesia)

Previous studies have shown the influence of intake calcium to the hardness of the enamel. To determine type of calcium, we measured the effect of various dietary calcium to the hardness of the enamel. Ninety six Californian White Rabbit's offspring were divided into four groups, with one group randomly designated as control. Four groups of those rabbits were fed with the following diet: group one: purified diet and calcium carbonate; group two: purified diet and dicalcium phosphate; group three: purified diet and calcium fluoride; group four: purified diet only (as control). The rabbits were sacrificed after ten, fourteen and eighteen weeks and the hardness of the upper incisors were tested using the Leitz Micro-Hardness Tester. The finding of the tooth enamel hardness of rabbits fed with purified diet and calcium carbonate is highest (ten weeks $\bar{X}$ = 257,86 ± 16,26; fourteen weeks $\bar{X}$ = 279,85 ± 20,33 and eighteen weeks $\bar{X}$ = 272,69 ± 16,94), followed by that with purified diet and dicalcium phosphate (ten weeks $\bar{X}$ = 232,54 ± 38,14; fourteen weeks $\bar{X}$ = 273,90 ± 21,67 and eighteen weeks $\bar{X}$ = 281,20 ± 19,06), while that with purified diet and calcium fluoride is the lowest (ten weeks $\bar{X}$ = 189,13 ± 60,24; fourteen weeks $\bar{X}$ = 185,00 ± 48,15 and eighteen weeks $\bar{X}$ = 155,05 ± 74,50). These differences were significant ($p < 0,05$) and tested by ANOVA. We conclude that the addition of various calcium to the diet of rabbit influence the hardness of the enamel. This study was supported by the Supersemar Foundation, Grant 240846.