

ular arch and is classified as a connective tissue (membranous) bone.¹ Nevertheless, some studies have suggested that the otic capsule is essentially a membranous bone, and the stapedial base, annular ligament (AL) and oval window are derived from the mesenchyme of the otic capsule.^{2,3}

Various studies have shown that the mandibular joint (MJ) is histologically similar to the periodontal ligament and vault sutural complex, because the processes of fibrillogenesis, osteogenesis and remodeling in their articular tissues are quite similar.^{4,6} In particular, some have observed that the articular cartilage of the MJ is covered with a force-shearing periosteal area fibrous tissue that shifts towards the synovial osteochondral junction (synovio-cartilage junction; transitional fibrocartilagenous zone) of the joint. The studies have observed that the periosteal area was composed of fibrous and subfibrous cell zones. Furthermore, the subfibrous cell zone was found to be responsible for histodifferentiation of both the fibrous cell zone and the osteo-chondro progenitor cell layer of the condylar cartilage, and therefore the periosteal area affected the remodelling activity and adaptive growth of the mandibular joint.^{4,7-11}

The stapediovestibular joint (SVJ) has been found to comprise a synostosis, synchondrosis, syndesmosis or diarthrosis in different species.¹²⁻¹⁷ Histology on the human SVJ has shown that the rims of the oval window and stapedial base are covered with hyaline cartilages, which are inserted and suspended with radially directed fibers of the AL.^{18,19} Physiological studies have indicated that vibrations of the tympanic membrane may be amplified 15-folds via the auditory ossicular chain and transmitted towards perilymph of the inner ear, and the AL acts as a kind of hinge to sustain stretching stress during acoustic oscillation. Some studies have found that the SVJ is also identical with the MJ, sternoclavicular joint, acromioclavicular joint, and cranial and facial sutures, in that they possess articulating surfaces covered with a fibrous layer that is physiologically remodelled throughout life.^{2,12-17}

Studies on the extracellular matrix (ECM) have observed that the tissues with the greatest tensile strength are those with the highest collagen content, although the flexible collagen fibrils lack elasticity

and the fibril orientation differs in various tissues.^{20,21} A fine structure study has indicated that collagen fibrils are axially oriented to transmit force and stressed that the distribution of fibril diameters is strongly correlated to the mechanical properties of the connective tissue.²¹ Conversely, besides collagen, the co-existent elastic elastin fibers (elastic system fibers; ESFs) have been found to be particularly abundant in sites that are frequently subject to specific and periodic stress.^{20,22-30}

In the present study, we examined the development of the ossicular chain, SVJ and MJ of foetuses at stages between 11 and 20 days of gestation (v.p.x.d: x days from insemination; full term = v.p.20.d) from pregnant mice and mice at stages between 0 and 28 days after birth (b.x.d: x days old). The fine structure study was in particular focussed on the articular ECM, and attempted to elucidate the elastogenesis causing adaptations for mechanical requirements in various joints at different developmental stages.

MATERIALS AND METHODS

Heads of Slc:ICR mice (Slc: Shizuoka Laboratory Animals Center, Shizuoka, Japan; ICR: Institute of Cancer Research) at stages between v.p.11-20.d and b.0-28.d were utilized in this study; each stage included five samples. The region containing the MJ and temporal bone (which contains the developing ossicular chain, labyrinth and SVJ) was dissected out and immersed in chilled (0-4 °C) fixative solution containing both 2.0% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate for 1 to 8 h. Samples were then rinsed for 24 h in chilled sodium cacodylate (0.1 M) buffer solution, postfixed for 1 to 2 h in 1.0% osmium tetroxide solution, then rinsed and demineralised in 0.5 M EDTA for 1 to 4 weeks. Samples were then rinsed for another 1 to 4 weeks in the buffer solution, dehydrated through a graded series of ethyl alcohol, substituted with propylene oxide, and embedded in Epon 812 using conventional methods. Frontal sections (1 µm thick) were prepared using glass knives mounted on an LKB 4800A Ultratome (LKB, Bromma, Sweden), and triple-chrome stained with 0.5% malachite green (80 °C; 60-100 s), 0.5%