Veterinary Functional Histology
Bone and Cartilage
Weisbrode
2008

OSTEOBLASTS; BONE MATRIX; INITIATION OF BONE MINERALIZATION.

I. Osteoblasts

What are osteoblasts? Osteoblasts are cells that produce bone and cover the surfaces of bone

Osteoblasts are of what stem cell line? Mesenchymal stem cell line

What do osteoblasts do? They sythesize osteoid and deposit mineral (hydroxyapatite) into the osteoid (osteoid = organic matrix of bone); they embed themselves in bone matrix to become osteocytes; they initiate bone matrix mineralization via matrix vesicles; they control initiation of bone resorption by osteoclasts and they interact with osteocytes to pump calcium out of bone (osteoblast-osteocyte osteoblast calcium pump). Details of all of these to follow.

II. Bone Matrix and its Mineralization

What is the matrix of bone composed of? The organic matrix (osteoid) is type I collagen and "Ground Substance"; the inorganic matrix is hydroxapatite (mineral). Mineralized osteoid is just called bone!

What is the nature of type I collagen? It is a protein that is produced and secreted by osteoblasts (and also fibroblasts) that is composed of a triple helix of amino acids. These helically arranged amino acids form the molecular basis of collagen known as tropocollagen molecules. These molecules are separated by each other by spaces (gaps) known as "hole regions". The tropocollagen molecules overlap

each other in a very orderly manner and are extensively cross-linked – this makes collagen a tough but flexible fiber.

Is there a function for the "hole (gap)" regions in collagen? The hole regions play a role in expanding the initial sites of mineralization of bone matrix. Details on this to follow.

What is lamellar bone? This is the term given to the pattern of bone matrix collagen deposition (parallel arrangement of collagen fibers) in normal adult bone. Adult trabecular bone and adult cortical bone is all lamellar. The collagen is deposited in 2-3 micron parallel layers. Each layer is called a called lamella.

If bone is not lamellar what is it? The alternative is woven bone. This is bone of the fetus and the bone in fracture repair or bone rapidly deposited in response to injury. In these situations the collagen is deposited in haphazard layers.

What are some of the biologically significant components of "Ground Substance" ? Noncollagenous proteins. These are proteins deposited by osteoblasts and left in the matrix. Examples are: growth factors (TGFβ; PDGF; BMP); glues (osteonectin; sialoprotein) that likely help bind mineral to collagen; helpers in initiation of mineralization (sialoprotein – possibly plays a role in mineral deposition in the hole regions between tropocollagen molecules); and useful indicators of bone formation (osteocalcin) but with no known function!!!

What is the mineral component of bone matrix called? Hydroxyapatite – consisting mostly of Ca and P.

How do osteoblasts initiate mineralization in bone matrix? Mineral is initially concentrated in finger-like cellular extrusions (into the osteoid surface) from osteoblasts called matrix vesicles. Similar cellular extrusions, also called matrix vesicles, occur at the level of mineralization of cartilage of the growth plate and are produced by chondrocytes. Matrix vesicles contain enzymes to cleave mineralization inhibitors naturally present in collagen matrix (why would these inhibitors be present in type I collagen????). Matrix vesicles also have surface membrane lipids to attract calcium and membrane electrolyte pumps to pass and concentrate Ca and P into the vesicle. Eventually the Ca and P concentration in the vesicle exceeds solubility and it forms a crystal that ruptures the vesicle and

allows mineral to now have access to the collagen matrix where it preferentially deposits initially in the hole regions between tropocollagen molecules. Eventually mineral precipitates in the entire matrix but leaves a VERY thin surface layer unmineralized beneath the osteoblasts; this layer is the lamina limitans.

III Osteocytes

What are osteocytes? Osteocytes are cells that reside within the bone matrix; osteocytes are derived from osteoblasts that became embedded in matrix during bone formation. Osteocytes live within holes in the matrix called lacunae and connect up with other osteocytes and overlying osteoblasts via slender cytoplasmic processes that extend through narrow canals in the bone called canaliculi.

What do osteocytes do? Osteocytes likely sense changes in movement of, and electrolyte concentration in, interstitial bone fluid. Fluid movement through canaliculi can cause electric currents called streaming potentials. In addition, physical disruption (eg. fractures) of canaliculi likely affect these potentials. Osteocytes are thought to be able to detect changes in these streaming potentials and send appropriate signals to the overlying osteoblasts. Osteocytes likely detect changes in electrolyte concentration by cell surface receptors. The osteocyte is thought to be able, when necessary for purposes of calcium homeostasis, to **precipitate** miniscule amounts of mineral onto the lacunar/canicular surfaces or **resorb** miniscule amounts of mineral from the lacunar/canicular surfaces. This deposition or resorption of mineral ultimately would affect blood calcium/phosphorus levels due to connection osteocytes to surface osteoblasts.

IV Osteoclasts

What are osteoclasts? Osteoclasts are multinucleated cells that resorb bone. They arise from hematopoietic stem line.

What controls osteoclastic resorption of bone? Most physiologic stimuli to resorb bone act through the osteoblast and not directly through the osteoclasts. Osteoclasts must be directed to resorb bone by adjacent osteoblasts under normal conditions of modeling and remodeling. Osteoblasts prepare the bone surface by releasing collagenases that remove the lamina limitans from the bone surface.

Osteoclasts attach to mineralized bone by integrins in their sealing zones that bind to RGD motif ligands (arginine-glycine-aspartate) in the matrix. The sealing zone is at the periphery of the cell and forms a ring of attachment to the bone. The resorptive process is kept within the ring "sealed off" from the adjacent bone. These RGD ligands in the matrix to which the iintegrins of the sealing zone bind are possibly within sialoprotein (or other noncollagenous proteins) in the ground substance. Osteoclasts will not bind to the matrix if the matrix is not mineralized. Osteoclasts will not bind to lamina limitans – what's up with that?!

How do osteoclasts resorb bone? Osteoclasts resorb mineral and matrix under brush border (modified region of the cells surface membrane). Acid resorbs the mineral. Acid is produced as carbonic acid in the cell by the action of by carbonic anhydrase (water and oxygen enzymatically together) and is secreted across the brush border. The matrix is dissolved by proteinases (cysteine, metallo) that are stored in lysosomes of the cell and secreted across the brush border. The polypeptides and amino acids from the hydrolyzed collagen and calcium and phosphorus and other salts from the mineral dissolved mineral are released locally. Howship's lacuna is the name given to the scallop-like cavity in the bone caused by the osteclast resorption.

V. Bone Development *in Utero*

What are the ways bone develops in utero? Bone can develop from an original cartilage precursor of mesodermal origin (endochondral bone) or can develop directly from a primitive neuroectodermal tissue with osseous potential (membranous bone). Endochondral bone is what happens in most bones of the body. In endochdonral bone, the primitive mesenchyme matures into a hyaline cartilage model of most of the skeleton. Initially this cartilage model is without separation between what will become the individual bones. Bone that develops directly from osseous metaplasia from condensed neuroectodermal tissue is called membranous bone. Membranous bone is represented by bones of the top of the skull and the horizontal mandible.

What is the benefit of one type of bone development over the other? When in the cartilage stage, cartilage model bone can grow faster (interstitially and appositionally). Membranous bone can only grow appositionally. Likely more importantly, only endochondral bone elongates via a growth plate. Can you think of why such bones grow in length from growth plates rather than growing from their ends?

VI Endochondral Ossification

What does endochondral bone look like BEFORE ossification takes place? The skeleton other than the horizontal ramus of the mandible and top of the skull consists of hyaline cartilage that arises from metaplasia of a condensed primitive fibrous mesenchyme. Apoptosis takes place in regions that are to become joints so that individual bones become apparent. The initial bone forms in the center of the diaphyseal region of the embryonic cartilage model of the bone and is called the primary center of ossification. The process is like that occurring at the growth plate (see below). The primary center of ossification expands towards the metaphysis leaving a band of cartilage to become the growth plate. The growth plate separates the elongating metaphysis from the epiphysis which has to develop its own center of ossification (secondary center of ossification).

VII Growth Plates (how bone grows in length)

What are growth plates? Growth plates are bands of hyaline cartilage designed to elongate bone. Growth plates at the metaphyseal/epiphyseal junction are called the metaphyseal growth plate or physis. Growth plates of the epiphysis are less obvious and consist of the rim cartilage between the bone of the epiphysis and the overlying articular cartilage. This growth plate is called the articular epiphyseal complex (AEC). Chondrocytes in growth plates are arranged in columns and cause the bone (be it metaphysis or epiphysis) to lengthen (expand is better term for epiphysis) by pushing the overlying tissue back in space. The pushing is done by the chondrocytes in the growth plate undergoing proliferation and hypertrophy. The "conversion" of the cartilage to bone is as follows: Cartilage matrix mineralizes (via matrix vesicles as above) -> chondrocytes die (apoptosis) –vessels invade from the periosteum and carrying osteogenic mesenchymal cells with them-> the lacunae of the chondrocytes are partially eroded (by whom?) -> thin mineralized cartilage matrix spicules remain -> bone is deposited on the remaining cartilage matrix spicules by the osteogenic cells brought in with vessels arising from the periosteum -> primary trabeculae are formed (bone on top of cartilage matrix spicule) -> osteoclasts develop in the marrow space from hematopioetic stem cells -> primary trabeculae undergo modeling to form secondary and tertiary trabeculae (reduction in number but thicker in structure).

VIII Appositional growth (how all bones increase in width)

How do bones grow in width? Bones grow in width by depositing new bone tissue on top of the outer surface of the existing cortex. This is done by the periosteum. The periosteum has a fibrous outer layer and an osteogenic inner layer. The inner osteogenic layer is capable of depositing new bone directly on top of the preexisting bone. This is done by metaplasia of the osteogenic layer from a fibrous tissue to an osteoblastic tissue – no cartilage intermediary is present.

What is the pattern of the bone deposited by the periosteum in appositional bone growth? Although ultimately cortical bone in the domestic animals is osteonal (details presented below), periosteal bone formed during rapid early growth usually does NOT take the form of osteonal bone. In larger animals that need to walk or run at birth, the periosteum deposits multiple thin laminae (laminar bone) oriented parallel to the cortex. The spaces between these thin laminae will fill in (compact) with a combination of woven and lamellar bone to form the cortex of the young animal (compacted laminae of bone which consists of a mixture of woven and lamellar bone but none of it arranged in true osteons). This compacted bone has yet to undergo primary remodeling for it to become osteonal bone. In smaller animals, the developing cortex is also derived from the osteogenic layer of the periosteum. It deposits bone in the form of thick anastamosing trabeculae of woven bone. The spaces between these trabeculae fill in (compact) with a combination of woven and lamellar bone. As in larger animals, true osteons are not formed initially.

IX Remodeling

What is primary remodeling? It is the resorption of the bone deposited during growth and replacing it with adult bone. This concept is most important in cortical bone in which the original bone is a combination of woven and lamellar compact bone but it is not osteonal. The definition of an osteon will come later.

How does remodeling take place? Remodeling of bone follows a set sequence of events: activation, resorption, reversal, formation.

What is ACTIVATION of the bone remodeling system? This process involves stimulation of inactive osteoblasts (also called lining cells) by substances like (but not limited to) PTH (parathyroid hormone). The inactive osteoblasts contract via activation of cytoskeleton and remove unmineralized collagen (lamina limitans) from the bone surface by release of collagenases. The activated osteoblasts secrete osteoclast differentiation factor [ODF] which binds to the receptor [RANK]

on osteoclasts and stimulates osteoclasts to RESORB bone. Osteoblasts can inhibit the effect of ODF by secretion of the soluble blocking protein osteoprotegerin. (Can you guess the bone appearance of ODF knockout mice or osteoprotegerin knockout mice?)

How does RESORPTION take place in remodeling? Resorption of bone initially is by osteoclasts by mechanisms described above (Section IV) with completion by mononuclear cells that might be of osteoblastic lineage (controversial).

What is reversal of remodeling? REVERSAL is the inhibition of resorption and initiation of formation at the resorption site. This is likely under the stimulation of noncollagenous bone matrix proteins such as the cytokine TGF beta.

What is the significance of the site at which reversal takes place? The site of reversal is called a cement line. The new bone that is deposited at the resorption site once the reversal begins is NOT in direct contact with the adjacent preexisting bone but is separated by a very narrow gap called a cement line. These lines are visible in routine histologic section (mineral removed and stained with hematoxylin/eosin) as a blue line. Cement lines are collagen poor, proteoglycan rich, and mineral rich sleeves that allow for slippage between remodeled units and possibly act as a sumps to dissipate microcracks.

How does FORMATION take place? Osteoblasts fill in the excavated space as described above (Sections I and II).

What is the purpose of Primary Remodelling? Primary remodeling converts the bone completed by the growth process to adult bone. The bone completed at the end of growth usually is not osteonal in the cortex and might be a mixture of woven and lamellar bone both in the cortex and in trabecular bone.

What is an osteon and a basic structural unit (BSU)? These are the names of the remodeling unit in cortical and trabecular bone respectively.

What is the shape of an osteon? It is a cylinder of bone composed of concentric layers of lamellar bone. The osteon is formed by a “drill bit” of osteoclasts that dig a tunnel in the bone. This tunnel is filled in concentrically by osteoblasts that leave

a small open core (the Haversian canal) in the center of the osteon vessels and nerves to have access to the cortical bone.

What is the shape of a BSU? It is a trench filled in with parallel layers of lamellar bone. It can be considered a longitudinal half of an osteon.

What is the purpose of primary remodeling? Primary remodeling likely improves the strength and flexibility of skeleton. The "internal" borders of BSUs and Osteons are demarcated by cement lines. Cement lines are collagen poor, proteoglycan rich, and mineral rich sleeves that allow for slippage and possibly act as sumps to dissipate microcracks. Osteonal bone is not found in small short lived animals like rats and mice (these species do not remodel in the same manner as larger mammals).

What happens to the skeleton after primary remodeling? Secondary remodeling continues throughout life with replacement of old bone by new bone (replace old BSUs and Osteons with new ones). In NORMAL remodeling there is no change in the shape or amount of bone produced.

What is the purpose of secondary remodeling? Likely it is to repair microdamage (remove microcracks before they become full fractures) in the bone.

X Modeling

What is modeling? Modeling is the change in shape or size of bone. The best example of modeling is normal growth.

What influences modeling other than normal growth? Modeling is the way bone responds to use. This could be normal or abnormal use. Bone's response to use is to form at sites of compression and resorb at sites of tension. The adaptation of the shape and orientation of bone to use is called WOLFF'S LAW (shape and orientation of bone will adapt to altered use).

How is bone able to detect use? Complicated and not fully understood. Likely includes effects of the following on bone cell activity: alterations in flow of blood; changes in piezoelectric forces (which are currents due to distortion of the

collagen lattice of bone); changes in streaming potentials (which are currents due to fluid flow through canaliculi); and deformation of stretch receptors (which alter calcium entry into cells).

What type of bone is formed in modeling? The bone is lamellar if the modeling is slow or woven if rapid (as in response to inflammation or trauma or neoplasia).

XI Cartilage (discussion limited here to hyaline cartilage of joints)

What are the components of articular cartilage? This cartilage consists of chondrocytes, type II collagen, ground substance composed of large proteoglyan molecules and water. The deepest layer in adult animals is mineralized (hydroxyapatite). Articular cartilage is avascular

How are chondrocytes different than osteocytes? Although each cell lives in a hole in the matrix called a lacuna, there is no space between the chondrocyte and the matrix as there is with osteocytes and its matrix and there are no cell processes extending from chondrocytes out into the matrix. There is no direct commincation between chondrocytes and communication between chondrocyte and the matrix is in the form of cell surface receptors which bind to collagen and proteoglycans in the ground substance of the matrix. Also unlike osteocytes which have no ability to divide, chondrocytes in mature cartilage have ability to divide but this ability is VERY limited.

What is the purpose of chondrocytes? Chondrocytes produce and maintain collagen and produce and maintain the ground substance of cartilage.

How are chondrocytes arranged in articular cartilage? Actually the very most superficial layer of articular cartilage is composed of fibrocytes with type I collagen Deep to this the cells are all chondrocytes (with type II collagen) starting with a tangential layer oriented about 45 degree angle to surface; a radial layer arranged perpendicular to surface and a deep layer of cartilage that varies in its appearance depending on the age of animal. In growing animals this deep zone is a growth plate called the articular epiphyseal complex (A-E complex). In adult animals this deep zone is completely mineralized cartilage and called the mineralized layer of articular cartilage. The "line" between the unmineralized and mineralized articular cartilage in the adult is called the tidemark.

There is no name for the junction between mineralized articular cartilage and subjacent bone. What keeps mineralized cartilage attached to the subjacent bone?

Where are chondroblasts? Chondroblasts (cells that cause cartilage to grow appositionally) are limited to the perichondrium which lines cartilage surfaces that are NOT weight bearing. This means weight bearing surfaces of joints no longer are able to grow appositionally. Since cartilage can grow interstitially chondroblasts are not as critical to continued growth of cartilage as osteoblasts are critical to continued growth of bone.

What are the components of the matrix of articular hyaline cartilage? As stated above there is a small superficial layer of surface type I collagen with little ground substance. Beneath this, the cartilage consists of type II collagen with VERY low turnover and very large proteoglycans with high turnover.

How are the collagen fibers arranged? The same orientation as the chondrocytes as described above. The very superficial layer (really fibrocytic in origin) is parallel to the weight bearing surface; the next layer is tangential to the weight bearing surface; and the radial and deep layers are perpendicular to the weight bearing surface.

What is the role of proteoglycans in articular cartilage? These molecules (eg. aggregan) are highly hygroscopic (bind water) and produce a hydrated gel keeping the collagen fibers separated.

What is the synovium? Synovium is the inner lining of the joint capsule. The outer layer of the joint capsule is dense fibrous connective tissue. The synovium consists of an intima (internal surface layer) of synoviocytes which is a discontinuous layer of cells that are either of macrophage phenotype and function or fibroblast phenotype but with specialized function of production of hyaluronan (hyaluronic acid) and lubricin (glycoprotein). There is a variable fibro-fatty stroma beneath the synoviocytes with numerous blood vessels.

What is Synovial Fluid? This is the liquid in the joint space that participates in the lubrication the joint and is responsible for providing nutrition and waste removal for the avascular articular cartilage. It is a filtrate of plasma enriched with proteoglycans from the fibrous synoviocytes.

How are joints lubricated? Joint lubrication is mostly by squeeze film (hydrodynamic) lubrication. A pressurized film of water is released from cartilage matrix when the joint is under load. Water returns to cartilage (due to the hygroscopic nature of its large proteoglycans) when unloaded. This in-and-out flushing action also provides nutrition and waste removal for the avascular cartilage. Boundary lubrication (surface lubrication independent of a fluid film) is likely due to lubricin which binds to the articular (and possibly synovial) surface to reduce friction. Hyaluronic acid of synovial fluid is no longer thought to function as a significant lubricant.

Synovial fossae are depressions of unknown function that are present on NON-weight bearing surfaces of articular cartilage. They are not present at birth. In horse are present by 3-5 months of age. They are not present in the dog and cat.