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A Review of Literature for Osteology: Cell Biology, Tissue Biology, and the Application of Synthetic Compounds for the Facilitation of Bone Tissue Repair

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Introduction

Bone is a dynamic matter that provides support, structure, mineral reserves, and stem cell reserves for the body. Important functions range from structural support for the body to roles in maintaining homeostasis. Structure and support for the body is the most obvious role, with the skeletal system as a whole providing a normal force for other tissues and organs to resist gravity. Protection is also inferred for tissues and organs from impacting forces, especially with axial bones covering vital organs in the thoracic cavity. Another function of bone includes the ability to store and release minerals when needed to maintain appropriate levels in circulation. Specifically the resident mineral of bone tissue, hydroxyapatite, is composed mostly of calcium and phosphorus. Calcium and phosphorus are vital in essentially every system in the body, from neurotransmitter release, to muscle contractions, to various biochemical processes, and so on. Bone has the ability to dissociated hydroxyapatite to release and provide these two elements to the body. Lastly, bone provides a location for the storage of stem cells, residing in bone marrow. These cells are vital to supplying new cells for the repair of tissue damage, resupplying the immunological system, generating erythrocytes for the circulatory system, and other functions requiring new cells.

A mixture of inorganic and organic matter provides essential properties to the tissue that allow for these functions to occur. Inorganic calcium and phosphate minerals combine with organic collagen fibers to form crystalline structures. The secretion, bonding, and breakdown of the mineral and fiber interactions are all controlled by cellular processes. The combination of solid, tensile, matter with living cells creates a solid material that constantly adapts to present needs of both the local environment and the body as a whole. This is accomplished by osteoblasts, osteoclasts, and osteocytes which either secrete, absorb, or control/sense extracellular matrix around them. The interaction between cells and abundant, tensile extracellular matrix create one of the most versatile, functional tissues in the body.

Bone cells also have the ability to develop and maintain extracellular tissue of varying structure. Examples include the macro-structure subtypes of cortical and trabecular bone tissue, and the micro-structure subtypes of lamellar and woven tissue. Cortical bone tissue has a rigid, orderly structured composition and provides good protection from impacting forces, while trabecular bone is porous in nature, transfers energy throughout effectively, and provides locations for the storage of bone marrow. Lamellar bone tissue has mineralized collagen fibers

that have been secreted by osteoblasts in a deliberate fashion. Finally, woven tissue involves the structure of mineralized collagen fibers that have been secreted into no particular orientation, with individual fibers bearing a random appearance of directionality.

The secretion, subsequent restructuring, and repair of bone are carefully regulated with intrinsic hormones, extrinsic hormones, and other various signaling molecules. This allows for specific structures of bone tissue to develop at the appropriate areas during growth and development. These various signaling pathways provide the basic machinery to allow for bone to have dynamic, adapting properties. Without careful regulation of bone cells and their activities however, the dynamic ability to provide an adequate structure and adapt to local and homeostatic needs would be lost.

Occasionally, damage does occur from natural forces upon bone from everyday activities and unnatural events like impacting forces. This creates fissures in the mineralized structure of collagen fibers and usually damages resident osteocytes. Depending on the magnitude of damage, resulting mechanisms either induce remodeling or inflammatory repair mechanisms to begin the generation of new tissue. Remodeling usually occurs through the use of basic multicellular units (BMU's), a combination of osteoclasts and osteoblasts working together. Larger scale damage uses the inflammatory response, which develops a temporary cartilage scaffold structure to provide intermediate support until new tissue can be secreted and mineralized.

When damage to bone tissue is large in magnitude, natural healing may take longer periods of time or even be unable to fully heal properly. Trauma and various diseases sometimes disrupt structure in extremely large proportions of bone. Waiting for the body to naturally repair this damage usually requires long periods without applying force to the bone, resulting in the loss of everyday care for themselves. Also, other physiological problems may arise, such as muscle degeneration and circulatory system complications. To increase both the speed and success rate of healing, human crafted materials have been applied to support bones for hundreds of years. An original idea of removing bone from another location or individual and using it to facilitate healing, known as grafting, was mentioned in mythology and documented several centuries ago. Bone grafting was a procedure beyond the medicine of that time, forcing early physicians to look elsewhere for the facilitation of bone tissue healing. This lead to the development of crafted materials in order to facilitate the healing of damaged bone tissue.

Early materials designed to facilitate bone tissue healing included the use of splints and casts for the immobilization of damaged bones. This simple, but effective idea has since evolved in the last century and a half to include materials implanted in vivo. Bone cements arose as the first in vivo materials followed shortly by plasters, both of which were injected or pasted where needed to facilitate the union of fractures. As with any new therapy, major complications arose with these products: such as problems toxicity, resorption into the body, lack of integration with existing tissue, and the formation of fibrous tissue at the application area. These problems drove new research to both refine the materials used in these products and the development of new materials.

Compounds such as tricalcium phosphate, bioactive glass, and glass ionomers were created in the last several decades to provide an intermediate scaffold that facilitated the bone tissue repair process. Eventually, even the natural resident mineral in bone tissue, hydroxyapatite, was synthesized in the laboratory to medical grade quality. The success of these materials derives from the ability to bind well with existing tissue, provide good intermediate support for the damaged area, and the capability for resorption by the body. These properties allow these compounds to avoid toxicity complications, bond well with existing tissue (avoiding fibrous scar tissue formation), and avoid antigenicity problems of allogenious bone grafts. Other compounds also used in modern orthopedics include metals like titanium, aluminum, and zirconium. Metals are usually exclusively reserved for screw implants to stabilize bone tissue, and at joints to resist the high physical strain of friction forces.

Future directions in modern medicine's search to provide effective orthopedic applications in the facilitation of bone tissue healing include areas of research in both stem-cell and gene therapies. Stem-cell therapy shows particular promise using the natural role of bone storing reserves of marrow. New applications and procedures under investigation include the extraction and incubation of stem cells from bone marrow, and transplant of these cells to the same individual or others. Stem cell application can also occur in combination synthetic scaffolding materials, providing implants with the ability to integrate, conduct new tissue growth, and provide the necessary precursor cells for growth. Gene-therapy on the other hand is under investigation for potential use in stem cell therapy. In the current models, various genes coding for extracellular signals inducing osteoblast formation and extracellular matrix secretion could possibly be inserted into the genomes of stem cells. The combination of new and older

developed products/procedures has lead to various therapeutic applications that individualize the orthopedic care patients can receive. This progress continues to expand the limits in the facilitation of healing bone tissue.

Cellular Biology

Introduction

Bone tissue is one of the few tissue types that rely heavily on interaction between a very solid extracellular matrix and cells. Mineralized tissue is secreted, regulated, and repaired extensively by local cells. Specialized cellular mechanics and signaling pathways are in place to control these processes. This ensures that bone tissue maintains its functionality through the rigors of physical forces experienced in everyday activities.

Three types of cells are directly involved in the growth, development, repair, and maintenance of bone: osteoblasts, osteocytes, and osteoclasts. Osteoblasts synthesize and secrete extracellular matrix, aiding in the formation of new bone tissue. Osteocytes, embedded throughout bone, regulate tissue mineralization and provide signaling mechanisms to convey information about the local tissue composition or damage. Finally, osteoclasts function to degrade and remove damaged and/or aged bone tissue. All three of these cells also exist in several different sub-types, giving rise to further specialized activities that vary with age, local tissue conditions, and signals.

A fourth cell type, chondrocytes, also play an integral role in bone tissue formation. Chondrocytes secrete extracellular matrix, forming cartilaginous tissue that eventually forms hyaline, fibrocartilage, or elastic cartilage. Hyaline cartilage is most important to bone, providing an intermediate, scaffold-like structure for eventually bone tissue formation in endochondral ossification. When hyaline cartilage is used to line bones and form joints it is commonly classified as articular cartilage. The other types of cartilage have limited involvement with bone tissue compared with hyaline cartilage. Fibrocartilage's major function associated with bones is the formation of tendons and intervertebral discs. Elastic cartilage has little direct association with bone outside of its close proximity in the formation of the larynx, pinna, and nose.

In the end, all four types of cells work together to allow for bone tissue to be a dynamic, evolving, properly developing structure. The constant interaction between extracellular matrix

and cells allows for tissue structure to be built, changed, or repaired without losing tensile strength or undermining homeostasis. Having a close, coupled relationship allows for a solid, mineral material composition that also has the capability to interact with other organ systems, provide information about its local environment, and creates mechanisms to maintain it. In the end the overall regulation of bone function and structure, as with any other tissue or organ, attributes to the individual cells composing it.

Osteoblasts

Osteoblasts are the major contributors in bone formation by synthesizing and secreting collagen fibers, regulating the calcification of bone tissue, and controlling the differentiation of other bone cells. Histologically, these cells are cuboidal shaped, have prominent endoplasmic reticulums, posse numerous secretory vesicles, large nuclei, and aggregate to form sheets at the sites of bone formation (Liu and Deng 2005). Osteoblasts can also be further sub-classified into three different sub-types identified based on anatomical position, stage of development, and function.

Osteoblasts originate from mesenchymal stem cells (MSC) derived either the mesoderm in embryonic development, stroma of bone marrow, or other circulating stem cells and give rise to multipotent progenitor cells (Hesslein 2005). From here multipotent progenitor cells bear the capability to differentiate into preosteoblast cells or into other types of cells, such as osteoclasts or immune cells. After differentiation, the preosteoblast sub-type of osteoblast cells can be found superficial to locations of bone formation. Extracellular matrix excretion does not directly occur with this type of osteoblast, but the production of collagen type-1 precursor molecules occurs readily. These precursor molecules only need post-translational modification to become mature collagen fibrils ready for future extracellular matrix secretion of osteoid tissue (Hall, Franz-Odendaal, and Witten 2006).

The second sub-type of osteoblast cells are known as active-osteoblasts: cells that actively participate in the formation of bone tissue. These cells possess large numbers of golgi, endoplasmic reticulum, and vesicles to provide the necessary machinery needed to secrete large concentrations of extracellular matrix/osteoid. This type is distinguished by the original expression of RANKL receptors. RANKL is a transmembrane receptor that projects to the

extracellular side of the cellular membrane and controls the differentiation of osteoclasts from precursor cells.

Under the tight regulation/control by hormones and other local signals, active osteoblasts generate osteoid tissue by synthesizing and secreting type-I collagen fibers in combination with other proteins. These fibers are laid down either a randomly or orderly fashion of multi-lamellar sheets depending on the tissue already in place. In woven bone formation collagen fibers are laid randomly onto cartilage or damaged tissue, allowing for faster mineralization and bone tissue generation. This creates scaffolding to provide immediate structure until future, orderly collagen fiber secretion and/or extracellular matrix mineralization can occur. Ordered, extracellular matrix secretion by osteoblasts on the other hand, develops lamellar tissue. This type has layers of fibers parallel to each other and perpendicular to the neighboring layers, creating a tissue with great mechanical strength (Liu and Deng 2005). The orderly structure now provides adequate scaffolding for minerals to enter and crystallize forming an even stronger structure.

Other non-collagenous proteins are secreted by active osteoblasts; present to help regulate tissue mineralization, cell adhesions, and collagen fiber polymer bonding. Non-enzymatic bonding between these proteins, calcium, and phosphate aid in the structural stability of the extracellular matrix. Several enzymes also enhance these bonds by facilitating covalent bonds between glutamine and lysine residues at the collagen fiber ends. Transglutaminase enzymes in particular, have the most influential role, facilitating the protein-mineral bonding in the mineralization process (Kaartinen et al. 2006).

During and after extracellular matrix secretion and osteoid formation, active osteoblasts have one of two fates if they survive the process: differentiate into osteocytes amongst the tissue, or progress through the formation process and return to the superficial bone surface. First, as extracellular matrix is secreted, some osteoblasts become surrounded and eventually are embedded amongst the tissue. This process leads to the eventual differentiation of these cells to osteocytes. The second fate occurs with active osteoblasts returning to the superficial bone surfaces and differentiating into the third type of osteoblasts: bone-lining cells or resting osteoblasts. These elongated, flat cells form a functional, monocellular epithelial barrier, on both the periosteal and endosteal bone surfaces. Histologically, they possess a thin, flat nucleus with attenuated cytoplasmic processes extending into bone tissue to osteocytes (Liu and Deng 2005).

The dendric connections between bone-lining cells and osteocytes are mediated via gap junctions. Together, the aggregate of these connections form the osteocytic lacuno-canalicular network system throughout bone tissue. This system is the ideal candidate for the sensory component for the mechanostat sensory theory: where forces upon the bone cause cytoplasmic flow that triggers the release of signaling molecules (Mullender 1997). Combined with other physiological changes amongst the bone tissue, signals can be transduced through this network to bone-lining cells. This paracellular signaling/recruitment is thought to be a key component in the initiation of either bone modeling or remodeling in the adaptation of bone tissue's structure (Pandaranandaka et al. 2008).

The layer of bone-lining cells/resting osteoblasts are interconnected via tight junctions, forming a functional barrier along the surfaces of bone tissue (Pandaranandaka et al. 2008). This barrier helps regulate and maintain ion concentrations between the tissue and interstitial fluid surrounding, creating ideal conditions such as mineral concentrations needed for bone modeling and remodeling. Also, the layer of bone-lining cells protects the tissue from irregular/premature osteoclastic absorption (Pandaranandaka et al. 2008). In order for bone modeling/remodeling to occur, this layer must be removed. Upon proper signaling for the initiation of osteoclastic absorption, the bone-lining cells contract their cell body via microtubules and begin secreting proteases to digest local bone tissue (Liu and Deng 2005). The end of the bone-lining cell's life cycle remains unclear. Possible outcomes include differentiating into mesenchymal stem cells, differentiating into preosteoblasts, undergoing apoptosis (a possible mechano-sensory mechanism), or activating into an active-osteoblast once more (Liu and Deng 2005).

Osteocytes

Osteocytes are the most abundant cell type amongst bone tissue, at a scale of around 95% of all cells present (Pallu, Rochefort, and Benhamou 2010). These cells are found embedded within abundant extracellular matrix in bone tissue. Osteocytes provide mechanisms to communicate information about the local tissue environment via the lacuna-canalicular network mentioned previously. The concentration of these cells varies with different types of bone tissue. Lamellar bone and cortical tissue types typically have more osteocytes per area of extracellular bone matrix than woven and trabecular types. Osteocytes in woven bone however are usually

much larger in size and to be present in slightly higher numbers in woven bone developed in the endochondral ossification process (Hernandez, Majeska and Schaffler 2004).

Osteocytes develop from active-osteoblasts during the bone tissue formation process. This occurs when osteoblasts become embedded, either by their own extracellular matrix secretion or by other active-osteoblasts (Reeve and Noble 2000). During embedment, osteoblasts begin to slowly lose their access to nutrients and signaling molecules provided by vascularity at the osteogenic front. This lowers the metabolic activity of osteoblasts, inducing the differentiation of these cells to osteocytes (Hall, Franz-Odendaal, and Witten 2006). During differentiation the developing osteocytes begin to change from a spherical to a stellate shape (Knothe and Tate 2004). The cell's cytoplasmic volume also redistributes itself into processes or dendrites that extend throughout the local tissue.

Young osteocytes facilitate the mineralization process by controlling the flow of nutrients through gap junctions in their dendric processes. In particular, minerals such as calcium and phosphate are regulated from extracellular fluid through osteocytes for deposit into gaps between collagen fibers for crystallization and hydroxyapatite formation (Liu and Deng 2005). Dentin matrix protein 1 is a possible candidate for the mechanism regulating mineralization in osteocytes. This protein is believed to change conformation in response to local tissue mineralization, signaling the completion of the process for the differentiation of the cell into a mature osteocyte (Shibui et al. 2008).

As an osteocyte matures, a decrease in metabolic activity results in organelle lost and an overall decrease in cellular volume (Liu and Deng 2005). Volume lost in the cellular body creates lacunae in the bone tissue and volume in dendric processes form canaliculi structures throughout the local tissue (Pallu, Rochefort, and Benhamou 2010). As this loss occurs, the dendric processes polarize their volume towards the nearest vascularity to maintain minor nutrient transport and exchange (Palumbo et al. 1990). A mature osteocyte also maintains gap junctions with neighboring osteocytes, allowing for nutrition to diffuse throughout the entire tissue (Pallu, Rochefort, and Benhamou 2010).

The life of an osteocyte ends with one of several possible fates: senescence, osteoclastic engulfment, or apoptosis (Knothe and Tate 2004). Senescence can occur from the strain forces experienced upon osteocytes, slowly breaking down their cellular structure over time. Areas of weakened bone may also fail structurally resulting in osteocyte damage and the release of

prostaglandins and other molecules to signal for repair. Osteocyte apoptosis can also play a key role in tissue maintenance, where aged or damaged osteocyte death creates signals for the modeling or remodeling of that tissue. However, the most common fate for an osteocyte is the engulfment by osteoclasts. During osteoclastic breakdown of tissue osteocytes are destroyed and their cellular components recycled to aid in the formation of new cells and tissue (2008).

Osteoclasts

Osteoclasts are present in bone to degrade and absorb bone tissue in the processes of modeling and remodeling. A mature cell can be identified by their large size, in comparison with other bone cells in the vicinity, and the presence of multinucleation. Osteoclasts also have polarized cell bodies, abundant vesicles and organelles. They are usually found in basic multicellular units throughout bone tissue, or bordering woven bone tissue (Gillespie and Quinn 2005).

An osteoclasts' life cycle begins with mesenchymal stem cells from the stroma of bone marrow, differentiated from other hemapoietic stem cells, or the mesoderm in an embryo. These cells differentiate and give rise to colony forming unit-granulocyte/macrophage (CFU-GM) which are stimulated by the ligand colony stimulating factor (M-CSF) to express the receptor activator of nuclear factor kappa B (RANK) (Schoppet and Hofbauer 2004). With RANK receptors the cells are now susceptible to the RANK/RANKL signaling pathway. Osteoblasts, dendric, and T-cells have the capability to produce this receptors' ligand RANKL, to control osteoclast differentiation. These cells also have the ability to produce osteoprotegerin (OPG) ligands, which directly inhibit RANKL-RANK binding (Laitala-Leinonen and Vaananen 2008). Combined with other stimulating and inhibitory factors like tumor necrosis factor (TNF), various transcription factors, and cytokine ligands, a system of feedback and control is present to govern the secretion and absorption of bone tissue (Boyle, Simonet and Lacey 2003).

Osteoclast differentiation continues with the polarization of the M-CSF cell bodies, to prepare for multicellular fusion. These cells fuse together to form multinucleated cells, which are subsequently attracted to bone tissue via chemotaxis (Gillespie and Quinn 2005). Signals for tissue absorption are provided by molecules derived via the modeling/remodeling control pathways discussed later. At the targeted tissue for absorption, the cells begin to further polarize and differentiate into active osteoclasts in preparation for the degradation of the tissue.

Osteoclastic absorption begins with the development of podosomes, adhesion structures composed of a cylindrical actin core surrounded by a domain of integrin, plaque proteins, and matrix metalloproteases (Addadi, Luxenberg, and Geiger 2006). Prior to active absorption these podosomes change their conformation using microtubules to aggregate into clusters, then rings, and finally into a belt-like structure at the periphery of the osteoclast. Here the podosome condenses, causing the separation of the plaque/protein outer ring and inner actin core. This segregation allows these structures to bind to the targeted bone tissue and begin forming a sealing zone (SZ) at the cell membrane lateral to the location of absorption (Addadi, Luxenberg, and Geiger 2006).

After adhesion, several other histologically classified domains along the cell membrane of the osteoclasts develop. The first includes the functional secretory domain (FSD): at the apex of the cell opposite to the ruffled border, that is mainly responsible for exocytosis of degradation products from the osteoclast. The other is basolateral domain (BD) which contains ion pumps and channels regulating ion and mineral concentrations for proper osteoclast functioning (Laitala-Leinonen and Vaananen 2008). Lastly, medial to the sealing zone is the ruffled border (RB), an area of cell membrane found in the resorption lacunae with convolutions that penetrate into the bone tissue to create maxim surface contact between the cell and bone tissue (Liu and Deng 2005). This area can be further sub-divided into two different areas of activity. The first is the peripheral fusion zone (FZ) which contains acidic vesicles and aiding in the degradation of tissue. The second is the central zone, where endocytosis of degradation products occurs. Together these areas of cellular membrane help form the resorption lacunae, an area between the osteocyte and the bone matrix where degradation occurs (Leeming et al. 2007).

Osteoclasticogenesis, begins originally with the breakdown of inorganic components of the bone matrix/tissue. Hydroxyapatite, the major salt mineral present in bone tissue, is a basic compound that is dissociated by osteoclasts with the secretion of acidic vesicles into the resorption lacunae (Laitala-Leinonen and Vaananen 2008). Acidic vesicle secretion is synergized using vacuolar ATPase pumps to excrete hydrogen ions and chloride-channel 7 pumps to removing counter chloride anions from the lacunae. The low pH environment amongst the bone matrix chemically liberates calcium and phosphate from hydroxyapatite breaking down the crystalline structure and exposing organic tissue for breakdown (Laitala-Leinonen and Vaananen 2008).

Organic tissue degradation and absorption begins with proteolytic cleavage involving cathepsins and matrix metalloproteinases (MMP's) (Liu and Deng 2005). The enzymes are produced in the golgi region in osteocytes, packaged into vesicles, transported to the ruffled border, and then secreted into the resorption lacuna (Schilling et al. 2006). MMP's start the degradation and cathepsins complete the final protein cleavages. In particular, the lysosomal proteinase cathepsin K is secreted in a large concentration and plays a major role in the breakdown of collagen fibers and other proteins present in bone matrix/tissue (Laitala-Leinonen and Vaananen 2008).

Removal of degradation products from the resorption lacuna occurs via two proposed mechanisms: detachment and reattachment of the osteocyte allowing products to diffuse out, or using active transcytosis through the osteocyte. Transcytosis occurs first with the products endocytosed into the osteocyte, followed by vesicular transport through the cell to the basolateral membranes, and finally exocytosed at the basolateral membranes and into the extracellular fluid (Mika Mulari et al. 1997). Recent research has shown that product removal due to diffusion from detachment does not occur nearly as common as transcytosis (Liu and Deng 2005).

Termination of osteoclastogenesis occurs via paracrine and systemic hormonal signaling. These molecules are essential to enforce stoppage after all targeted bone has been dissolved and removed. Improper bone tissue/matrix digestion by osteoclasts leads to many disease states such as osteoporosis. Locally, a possible method for termination may occur from molecules derived from tissue degradation. As bone tissue ages, like all other tissues molecules slowly begin to isomerize. In particular collagen type-I, the major organic constituent of the tissue, slowly isomerizes overtime after original synthesis. Using a ratio of isomerized to non-isomerized strains in an area of tissue a biochemically "age" can be derived by osteoclasts as these proteins are released during osteoclastogenesis (Leeming et al. 2007).

Local calcium concentrations may also play a role in osteoclastogenesis termination. As calcium ions are removed from local bone tissue and endocytosed by the osteocyte, they bind to intracellular receptors. The proposed pathway involved includes PI3K activation with G-protein intermediates to create the glycoprotein CSF-1. CSF-1 then is thought to ultimately induce motility through unknown mechanisms (Blair and Zaidi 2006). Interestingly CSF-1 involvement with macrophages induces differentiation and return of motility. With macrophage and

osteoclast linings deriving from a common predecessor this pathway's involvement in osteoclastogenesis termination is very possible (Stanley 1996).

Chondrocytes

The growth, development, repair of long bones occurs through cartilage formation, followed by extracellular matrix deposit and bone formation by osteoblasts. Central to this process are chondrocytes, cells that differentiate and produce cartilage which form a scaffolding structure for future bone formation. Chondrocytes are the only resident cells in the lacunae of the cartilage located in the epiphyseal plate of long bones or soft callus during bone tissue repair. These cells possess a round cellular body shape with one to several nuclei (depending on species), abundant golgi bodies, elongated mitochondria, and reserves of glycogen. A chondrocyte can exist in one of several different stages of maturity, identified histologically by their location relative to eventual hyaline cartilage development (Stockwell 1978).

Since hyaline cartilage is mostly composed of water (60-80%), chondrocytes have developed mechanisms to control their positioning and regulate osmotic gradients across their membranes (Shaw and Kheir 2009). Cellular orientation is maintained via β1 integrin receptors binding with the glycoprotein fibronectin dispersed amongst the collagen fibers in the extracellular matrix (Mehlhorn et al. 2006). Osmolarity is controlled using a pericellular matrix composed of proteoglycans and the glycoamine hyaluronan (Shaw and Kheir 2009).

The life-cycle of a chondrocyte begins with the recruitment of mesenchymal stem cells from bone marrow and developmentally from either the neural crest (cartilage of skull) or the mesoderm layer of an embryo (Lin et al. 2005). These cells are then induced to condense to form a layer of chondrocyte progenitor (chondroblasts) cells at a targeted location (Tsumaki 1999). Signaling growth factors induces individual cells amongst the condensed layer to further differentiate into chondrocytes, which enlarge and divide into two daughter cells that occupy a single lacuna. Primary cilium then rotate these cells against one another in their shared lacunae, moving from the lateral positioning characteristic of hyaline cartilage to a vertical orientation. This creates the visible columnar structure of chondrocytes found in the epiphyseal plate (Morales 2007). This collective area of proliferating chondrocytes at the tissue level is known as the proliferative region.

Following proliferation chondrocytes continue to elongate and begin to secrete collagen fibers and proteoglycans. In particular, these cells secrete mostly collagen type-II fibers along with a high concentration of the sulfated proteoglycans aggrecan (Beier 1999). The protein matrilin-1 is also secreted, aiding in the extracellular structure creating extra bonds between collagen fibers and proteoglycans (Ehlen et al. 2005). Together chondrocytes and the secreted constituents develop and create condensed hyaline in callus formation and articular cartilage in the anatomical pre-hypertrophic zone of the epiphyseal plate.

As the chondrocytes matures (and are forced distally away from the peri-articular plate in endochondral ossification) they begin to become hypertrophic and secrete collagen type-X fibers instead of collagen type-II (Liu and Deng 2005). Collagen type-X fibers bind to proteins in the cartilage; reorganizing existing type-I fiber structures into hexagonal lattices. This prepares the cartilage for eventual mineralization (Shaw and Kheir 2009). In addition to collagen, hypertrophic chondrocytes also secrete alkaline-phosphatase filled vesicles into the extracellular matrix (Stockwell 1978). The alkaline secretions are necessary to create a basic environment for the chemical reaction of apatite crystal formation and the subsequent mineralization of the extracellular matrix. These cells also secrete several signaling molecules; controlling the differentiation of younger chondrocytes and stem cells.

The life-cycle of a chondrocyte ends in normal circumstances with apoptosis. Before this occurs, these cells secrete matrix metalloproteinases (MMP's) to aid in degradation of the extracellular matrix. This creates space for angiogenesis to allow vascularity to enter the tissue in preparation for bone modeling/remodeling (Umlauf et al. 2010). Apoptotic cell death occurs by the increased expression of caspase and the decreased expression of bcl-2, controlled by a combination of signaling factors mediated by the p38 MAPK pathway (Lotz et al.2001) (Adams and Cory 2007).

Tissue Biology

Introduction

At the tissue level of bone, extracellular matrix greatly outnumbers the total composition of bone versus cells. However, this tissue is created and controlled by bone cells that even secrete specialized types of extracellular matrix resulting in different types of bone tissue. The creation of bone begins with a connective tissue template, usually hyaline cartilage. Formation occurs either by intramembraneous ossification or endochondral ossification, depending upon the

type of bone and/or the age of the individual. Intramembraneous ossification uses stromal connective tissue to form bones directly while endochondral ossification occurs with a cartilage template that is eventually mineralized, degraded by osteoclasts, and remodeled by osteoblasts with osteocytes.

The original bone/osseous tissue formed without ordered structure (with collagen fibers in non-specific arrangements) is known as woven tissue. Through the process of modeling or remodeling this tissue is eventually replaced with an ordered structure of collagen fibers to form lamellar bone tissue. A second classification for tissue types is also present, focusing on a higher-scale organization. Woven tissue can be secreted by osteoblasts in crescent-shaped packets or several layers of extracellular matrix stacked upon one and other. The same is true of lamellar tissue, but since they posse ordered structure of collagen fibers, the orientation of the layers can be controlled. Lamellar tissue can form organized packets or layers where the directionality of collagen fibers can be varied from around 90º to 30º from one layer to the next. When either woven or lamellar tissues are organized into crescent shaped "packets", they form the macro-structure of trabecular bone tissue. When either type is simply layered they form the macro-structure of cortical bone tissue.

Cortical bone tissue forms a "shell" around long bones and provides strength against impacting forces. The orderly, secondary bone structure with lamellar tissue provides these properties. Trabecular tissue is usually found deep to cortical in long bones, and forms the main composition of flat and cubodial bones. The porosity of trabecular bone allow for the storage of bone marrow while providing structural strength. Other names for trabecular bone include "spongy-bone" or "cancellous bone". Together various combinations of woven and or lamellar bone organizations allow for different types of bones and bone structure. From original development through an individual's life, all types are integral in the overall functionality of bone and the body.

Bone Tissue Growth & Development

Bone tissue in newly born infants is formed via intramembranous ossification, forming flat, compact bones. As the infant grows, endochondral ossification occurs contributing to the longitudinal growth, most notably in the long, appendicular bones. Intramembranous ossification forms the compact cortical bone "shell" and certain bones of the cranium such as the jaw and facial bones (Liu and Deng 2005). Specifically the mandible and other facial bones

derive with mesenchymal connective tissue from the neural crest, while other bones derive from the mesoderm layer of the embryo (Smith et al. 2009). In this process, bone tissue is formed directly without prior cartilage scaffolding, but instead from fetal mesenchymal stem cell tissue. Stem cells from the mesenchyme then condense and differentiate into 2-3 layers of osteoblasts. These osteoblasts then form woven trabecular bone tissue that is eventually replaced with lamellar, cortical or trabecular bone tissue (Ferretti, De Pol, and Palumbo 2003).

Endochondral ossification is an osteogenic process in which bone tissue is formed from a cartilaginous template. This process forms the bodies of axial bones like the vertebra and longitudinal growth of appendicular long bones like the femur (Liu and Deng 2005). Ossification begins with the differentiation and proliferation of chondrocytes which produce cartilage, as described previously. The chondrocytes become hypertrophic as they move away from the cartilage anlagen and begin to secrete different collagen fibers and alkaline phosphatase, aiding in mineralization (Shaw and Kheir 2009). This mineralization partially degrades the chondrocytes and allows for vascular invasion which is induced by the release of vascular endothelial growth factor (VEGF) (produced by hypertrophic chondrocytes) (Ortega 2004). Finally, osteoclasts transported by the circulatory system during vascular invasion begin to further degrade the extracellular matrix. This allows for osteoblasts to enter and begin secreting woven bone tissue, which is later replaced with lamellar tissue (Tatarczuch et al. 2008).

Cortical Bone Tissue

Cortical bone is the primary bone tissue type, contributing to 80% of skeletal tissue mass in adult humans (Liu and Deng 2005). This tissue creates a semi-solid “shell” that covers the superficial surface of entire bones and is most abundant in the diaphysis region of long bones. Cortical bone tissue is composed of subscale structures from the nanoscale to whole tissue, with each playing an integral role in the overall function and architecture. These structures can be divided using informal scales in meters as follows: at the sub-nanoscale are collagen fibers and minerals, lamella/woven structures at the nanoscale, osteons at the sub-microscale, and structures with multiple osteons at the microscale (Kachanov and Sevostianov 1998).

At the sub-nanoscale tropocollagen are laid in the form of triple-helix type-I collagen fibers, secreted by osteoblasts. Transglutaminase enzymes connect the fibers to each other at their ends. Other non-enzymatic, chemical reactions connect the fibers to each other and other

present proteins (such as fibronectin, osteopontin, and bone sialoprotein) at their longitudinal sides (Kaartinen et al. 2006). During osteogenesis gaps develop in between the collagen fibers and other proteins present in the tissue. Osteocytes regulate the influx of minerals into these gaps via nucleation, and eventually fill them with high concentrations of calcium and phosphate combining to form crystallized hydroxyapatite minerals (Jasiuk, Hamed and Lee 2010). Together mineralized collagen fibers create the basic functional structure of cortical bone tissue.

Next, at the nanoscale of structure the mineralized collagen fibers may be organized into either lamellar tissue or woven tissue. Lamellar bone tissue is organized with mineralized collagen fibers in parallel to each either, forming lamella sheets. These sheets are then layered onto each other either parallel to the previous layer or at around 30° to previous layers, the latter of which form cylinders for eventual osteon formation (Weiner 1999). The organized orientation of these fibers (in particular the cylinder structure) allows for increased tensile strength over a wide range of loading angles that bone tissue experiences (Peterlik et al. 2006). The presence of lamellar tissue structure is important for maintain strength in bone tissue, especially in the long bones in the epiphysis region.

The second sub-type of tissue characterization at the nanoscale level includes woven bone tissue. This tissue is generated much more quickly, has a less organized structure of mineralized collagen fibers, and forms no higher-level structures in cortical tissue. Research points towards the possibility of different types of osteoblasts secreting either woven or lamellar tissue, but has yet to uncover conclusive data on this matter (Gorski 1998). Woven tissue is secreted by osteoblasts when bone tissue is needed immediately, such as in fetal development (human cortical bone before the age of 4 is composed of mostly woven tissue), the beginning stages of endochondral ossification, or after a fracture/wound (Smith 1960). Structurally, collagen fibers in woven tissue lack the highly-ordered orientation of lamellar tissue, with fibers present in random orientations (Jasiuk, Hamed and Lee 2010). With collagen fibers lacking an ordered structure, the porosity of woven tissue is much larger than that of lamellar. It has been proposed that this porosity allows for a higher concentration of minerals to enter and to increase overall bone mineralization (Su 2003). Eventually, woven bone structure serves as a scaffold for osteoblasts and osteoclasts to return to remodel the tissue into lamellar structure (Liu and Deng 2005).

While the woven tissue has no higher level structural organizations in cortical tissue, lamellar on the other hand can be organized into osteons at the sub-microscale level (Kachanov and Sevostianov 1998). An osteon is a cylindrical structure about 250μM in diameter and 1cM long, formed from layers of (cylindrical structured) lamellar tissue (Jasiuk, Hamed and Lee 2010). Amongst the layers of lamella are spaces known as lacunae, which are longitudinally oriented on a plane tangent to the radial direction of the osteon (Ascenzi, Kabo and Andreuzzi 2004). Osteocytes are housed inside these lacunae and provided important maintenance and signaling functions for the tissue. Transversing the core of the osteon cylinder is a Haversian canal which houses blood vessels, lymphatics, nerves, and connective tissue. These canals are continuous throughout bone via Volkmann canals, which usually run perpendicular to the radial direction of an osteon's lamellae (Jasiuk, Hamed and Lee 2010). Lacunae, Haversian canals, and Volkmann canals are all interconnected by many smaller canaliculi openings (Liu and Deng 2005). With these connections nutrients and signals can be transferred throughout the mainly solid overall bone tissue.

Finally, at the microscale level if cortical bone tissue are the lamellar structures of circumferential lamellae, multiple osteons, and interstitial lamellae. Circumferential lamellae are not organized in the cylindrical osteon structure, but instead arise from mineralized collagen fibers that are organized in perpendicular sheets. These lamellae are secreted from osteoblasts at the periosteal surface of bone. The formation of these "sheets" create the most superficial layer in the "shell" of cortical tissue that circumscribes whole bones or new bone tissue in growing bones.

Multiple osteons are an important characteristic of cortical level bone, found in two types in the tissue: primary or secondary. Primary osteons form in newly mineralized circumferential lamellae via blood vessels transversing throughout, allowing osteoblasts precursors to migrate to these locations and begin secreting extracellular matrix. Osteoblasts begin secreting cylindrical lamellae in concentric layers around the blood vessels (Locke 2004). Together circumferential lamellae and primary osteons are known as primary bone.

Secondary osteons arise via bone tissue remodeling; existing osteons and lamellar tissue are absorbed and replaced. With the aid of various signaling mechanisms osteoclasts absorb older or damaged tissue creating resorption cavities: openings that clear room for vascularity and basic multicellular units to enter (Ascenzi, Kabo and Andreuzzi 2004). As new lamellae tissue

fills these openings, secondary osteons are created. The border between old and new tissue can be distinguished by circumscribing cement lines: areas of high mineralization and low collagen content (Holmes et al. 2005).

Leftover tissue from older osteons that have only been partially absorbed makeup the classification of interstitial lamellae (Martin, Burr, and Sharkey 1998). This tissue can be found sporadically throughout cortical bone tissue, but still bears lamellar structure and thus still provides good structural strength. Together these sub-structures create the overall cortical bone type, providing strength and protection for bones. The careful architecture and constant modeling and remodeling activity ensure that cortical bone tissue can withstand physiological stress. Without these properties, this tissue would not be able to survive the impact and tensile forces experienced in the diaphysis of long and the covering of other bones.

Trabecular Bone Tissue

Trabecular bone, also known as spongy bone, is a porous bone tissue that occupies cuboidal ones (i.e. vertebrae), flat bones, and the inner regions of long bones (Martin, Burr, and Sharkey 1998). Compared to cortical tissue, this type contributes to only 20% of skeletal mass (versus 80% for cortical), but is seven-fold more porous (Locke 2004). This allows for functions beyond simple structural support to include homeostatic roles: such as the storage of calcium and phosphate for the body and the storage of bone marrow. The sub-structure units of trabecular bone are nearly identical to that of cortical at the sub-nano and nanoscales, but begin to differ at the sub-microscale and microscale levels (Majumder and Mazumdar 2007). Overall, the structure allows for this tissue to withstand large amounts of stress with only a small amount of actual mass (Sidorenko et al. 2008).

Structurally, at the sub-nanoscale level trabecular bone tissue has the same arrangement of mineralized collagen fibers as cortical tissue. However new research underway aims to examine a possible difference in the orientation of apatite crystals between cortical and trabecular tissue (Hong, Hong, and Kohn 2009). Moving to higher level structures of trabecular tissue, these fibers are arranged at the nanoscale into either woven or lamellar tissue (just as with cortical bone tissue). Lamellar tissue differs slightly in trabecular tissue with the absence of concentric sheets; lamellae are instead stacked only perpendicular orientation to adjacent layers (Weinans et al. 2006).

Next, at the sub-microscale level the structure begins to differ greatly from that of cortical bone tissue. While woven tissue has no specific higher level structures beyond the sub-microscale level, lamellar tissue sheets are arranged longitudinally to create trabecular packets. Trabecular packets are unique crescent shaped aggregates of lamellar tissue packed densely together. These packets can then combine together to form slightly larger structures known as trabeculae, which can be classified as either type 1 or type 2. Type 1 trabeculae are formed by osteoblasts that replace woven bone tissue that was original formed through endochondral or intramembraneous ossification, while type 2 are formed by the bone tissue remodeling process (Kragstrup and Kragstrup 1983).

The overall macroscale structure of trabecular bone tissue lacks the vascularity provided by osteons. Instead nutrients are derived from the rich network of vessels transversing the porosity and marrow throughout trabecular bone (Liu and Deng 2005). Nutrient exchange is further facilitated with the presence of larger and more abundant lacunae with more osteocytes than in cortical bone (Hong, Hong, and Kohn 2009). This allows for an increase in surface area between osteocytes and surrounding tissue. When combined, the large porosity of trabecular tissue with increased nutrient exchange, and the presence of local bone marrow allows for trabecular tissue to undergo constant bone remodeling. Since this type of bone tissue is subjected to constant physical strain, remodeling and rebuilding is needed to allow this tissue to remain structurally adequate (Locke 2004).

Homeostatic Bone Remodeling, Damage, Repair, and Controls

Introduction

To ensure the integrity of bones, several mechanisms are present to control structuring, restructuring, and repair. Modeling and remodeling are two of these mechanisms, constantly providing bone with the structure needed to meet the stresses and homeostatic roles required. Modeling involves the addition or removal of tissue, while remodeling involves both mechanisms together. Osteoblasts, osteocytes, and osteoclasts are the cellular components that enable these processes to occur.

With the capability to restructure bone tissue comes with the potential for problems to occur in the course of these processes. Structural deficiencies may develop due to adequate tissue growth/replacement or the proliferation of excess tissue/extracellular matrix/cells. These problems lead to the common diseases like osteoporosis and bone tumors, which have a debilitating effect upon the overall structure of bone. To avoid such complications, several built in signaling pathways are present in order to ensure that growth, modeling, remodeling, and repair occur appropriately. Local and extrinsic controls exist allowing for the actions of bone cells to couple with local and body-wide conditions.

Inevitably, complications or damage to bone tissue do occur commonly in one form or another. Whether damage derives from disease or outside impacting forces, mechanisms are also available to repair and replace damage in a timely manner. Nano-scale level damage occurs frequently and forms structural deficiencies known as microcracks, small structural failures that can signal for bone remodeling. Larger scale damage involves inflammatory responses and fibrous connective tissue formation. Eventually, in normal circumstances, the connective tissue is replaced with hyaline cartilage by chondrocytes, undergoes ossification, and is eventually remodeling to an appropriate type of bone tissue for that location.

Bone repair, as with modeling and remodeling, is also closely regulated with various signaling pathways. This ensures proper tissue regeneration and avoides the formation of tumors (in normal conditions). In the end, the careful regulation of cells controlling bone tissue is needed to ensure these dynamic, solid organs bear the appropriate composition. With several dozen signaling pathways deriving both intrinsically and extrinsically, bone also contributes and responds to the needs of the organism as a whole.

Bone Tissue Modeling & Remodeling

Bone tissue modeling and remodeling are mechanisms that adapt bone structure and properties to both mechanical and non-mechanical stimuli to ensure long-term structural functionality. The terms “modeling” and “remodeling” are used in conjunction with the analogy to changing the structure of a building. Modeling occurs when a new part is added as an addition or an old part is removed without replacement, while remodeling occurs when a section is removed and a replacement is added. These processes both are vital to the proper structure of bone. Bone modeling occurs mainly during bone growth during childhood. Remodeling occurs

constantly in bone tissue, even under normal physiological conditions, to ensure appropriate structural strength (García-Aznar et al. 2009).

Bone modeling can be divided into two processes: formation drift, with the addition of tissue by osteoblasts and resorption drift, with the removal of tissue by osteoclasts. Each process occurs with either osteoblasts or osteoclasts working independently to change a growing bone's size, shape, or to repair (Seeman 2009). Modeling helps assure that the proper bone tissue architecture is developed for that specific bone's location and strain. After bone growth in the body has subsided in adulthood, bone modeling is rarely found in normal conditions (Martin, Burr, and Sharkey 1998). The controls for modeling match that of remodeling, which will be discussed shortly.

Bone tissue remodeling is responsible for adapting and repairing minor deficiencies in bone in response to both mechanical and non-mechanical stimuli. This process also includes woven bone tissue replacement by lamellar, the renewal of all bone tissue over time, and the restructuring of deficient bone architecture (Liu and Deng 2005). Remodeling is accomplished by the coupling of osteoblastic bone formation and osteoclastic resorption. The close coupling of physiological controls for both of these processes ensures a balance between the two is found.

Basic multicellular units (BMU's) made of around a dozen osteoclasts and hundreds of osteoblasts are responsible for the process of remodeling. The osteoclasts form a "cutting" hemicone at the front of the unit with osteoblasts forming an elongated "closing" cone behind along with a source of vascularity to exchange nutrients. The unit creates a "refilling tunnel" through cortical tissue and a "refilling trench" through trabecular bone tissue (Cooper et al. 2006). A BMU's lifecycle/the remodeling process can be divided into three different stages: origination, progression, and termination.

Origination occurs with the formation of BMU's at either the endosteal, periosteal, or Haversian canal surfaces (Jilka 2003). Formation of the unit is induced by either hormones from outside of the skeletal system (such as parathyroid hormone or estrogen), local cytokines, or local growth factors in response to structural, metabolic, mechanical, and non-mechanical requirements (Parfitt 2002). These signals bind mesenchymal stem cells from the connective tissue layer lining the bone surfaces or circulating in blood vessels and induce recruitment and differentiation. These stem cells then differentiate into the osteoclasts and osteoblasts that form and populate the BMU (Parfitt 2002). The same initiating signals also induce the bone-lining

cells to contract and expose the bone surface. Osteoclasticogenesis then begins at the front of the BMU, forming a hemicone shape that "cuts" through bone in the form of resorption trenches (trabecular tissue) or resorption cavities (cortical tissue) (Martin 2007).

Progression through bone tissue occurs with osteoclast reabsorbing tissue at the front of the unit's movement. Osteoclast survival and regeneration is maintained by the vascular supply to the unit, continuously bringing nutrients and new progenitor cells for differentiation (Jilka 2003). These progenitors also differentiate into osteoblasts, which lag behind the osteoclastic front and secrete new lamellar bone tissue in the histologically classified area of reversal phase (Parfitt 2002). The direction of the unit as a whole is controlled by the concentration of new osteoclasts attaching to the targeted bone tissue. The mechanisms which recruit these osteoclasts and ultimately control direction are currently unknown, although osteocytes are widely cited as producers directing this signaling (García-Aznar et al. 2009).

Termination occurs when the death/apoptosis of osteoclasts at the BMU resorption front are not resupplied with new cells to continue the process. Thus, factors that inhibit osteoclast differentiation serve as an important control for BMU activity. During termination however osteoblasts will continue to secrete lamellar tissue until the void created by osteoclasts at the front of the unit is completely filled (Jilka 2003). From here, the BMU begins to disappear and the remaining osteoblasts differentiate to quiescence, bone-lining cells.

Control of Bone Tissue Growth, Modeling, Remodeling

The regulation of bone growth, modeling, and remodeling is integral in maintaining proper tissue structure and function. Anomalies in these processes lead to several disease states that can lead to overall growth abnormalities, brittle bones, or even upset the homeostatic mineral balance in the body. Bone's importance for growth and homeostatic regulation for the body as a whole also means that changes in structure are open to influence from systemic signals/hormones. These changes are further regulated and tuned using local paracrine signaling. While some of these signals bind directly to intracellular or extracellular receptors, others activate secondary pathways using the OPG/RANKL/RANK regulatory system, discussed in detail shortly.

Important hormones involved in bone tissue growth, modeling, and remodeling include growth hormone (GH), insulin growth-factor 1 (IGF-1), parathyroid hormone (PTH), 1,25-

dihydroxyvitamin D3, and Calcitonin (Liu and Deng 2005). GH, produced in the anterior pituitary gland, primarily affects longitudinal bone growth by stimulating mesenchymal stem cells to differentiate into chondrocytes during endochondral ossification. Other effects may include serving as a biomarker for tissue remodeling and controlling mineralization (Salles 2006). IGF-1, produced in the liver (stimulated by GH), stimulates chondrocyte maturity and hypertrophy during longitudinal bone growth during endochondral ossification (Yakar 2002). PTH production occurs in the parathyroid glands and acts to increase the concentration of calcium in the circulatory system. Calcium is released from bone tissue via osteoclastogenesis, stimulated indirectly by PTH via osteoblasts in the OPG/RANK/RANKL pathway (Reeve and Poole 612-617). 1,25 dihyroxyvitamin D3 is a steroid hormone produced in the kidney from vitamin D that facilitates osteoclastogenesis (via the OPG/RANK/RANKL pathway) and increases calcium absorption in the small intestine providing adequate concentrations for bone tissue formation (Oreffo 1995). Finally, calcitonin produced in the parathyroid gland, directly binds to osteoclasts to inhibit bone resorption and induces chondrocytes to increase extracellular matrix production via secondary mechanisms (Christiansen et al. 2007).

Local factors involved in the control of bone growth and remodeling include interleukin-1 (IL-1), macrophage colony stimulating factor (M-CSF), interleukin-6 (IL-6), interferons, transforming growth factor β (TGFβ), and bone morphogenetic proteins (BMP's) (Liu and Deng 2005). These signals are produced by a variety of cells in the bone tissue, periosteal connective tissue, chondrocytes, and bone marrow. IL-1, a cytokine found in two forms (alpha and beta), stimulates osteoclast differentiation, proliferation, and activity by mediating the expression of the RANK ligand (Lee et al. 2010). M-CSF is a cytokine produced by stromal cells, induces mesenchymal stem cells to become motile and differentiate into osteoclasts for bone absorption (Matsuzaki et al. 2000). M-CSF also shows some inhibition effects for the differentiation of osteoblasts (Zaidi et al. 2001). The cytokine IL-6 is produced by normal bone cells (mainly osteoblasts) in response to PTH and 1,25 dihyroxyvitamin D3 to facilitate the differentiation of osteoclasts from progenitor cells (Liu and Deng 2005). Interferons are proteins produced by immune cells to fight infection, but have also recently been shown to inhibit osteoclastogenesis. Mediated using secondary mechanisms, these signals inhibit transcription factors that control the RANK receptor expression on osteoclasts, thus inhibiting their ability for stimulation via the OPG/RANK/RANKL regulatory axis (Weinstock-Guttman et al. 2009). TGFβ is a protein that is

released from extracellular matrix in bone tissue upon degradation. These proteins then induce the differentiation of mesenchymal stem cells into osteoclasts, but inhibit synthesis of acidic vesicles and mellaproteinases in osteoclasts slowing the resorption process (Lovibond and Fox 19). Bone morphogenetic proteins (BMP) are locally produced by mostly osteoblasts and sometimes chondrocytes. Several dozen types have been discovered and individual effects are still under investigation. BMP have commonly been found to induce osteoblast differentiation from mesenchymal stem cells, and are increasingly serving as good therapeutic applications in orthopedic medicine (Liu and Deng 2005).

Finally, receptor activator of NF-κB ligand (RANKL) a tumor necrosis factor (TNF) family member has a key role in the local regulation of bone modeling and remodeling. RANKL is a transmembrane bound glycoprotein cytokine, expressed by osteoblasts, osteoblast precursor cells, some immune system cells, and skeletal muscle cells (McCarthy et al. 2009). The receptors for RANKL include receptor activator of nuclear factor kappa B (RANK) and osteoprotegerin (OPG). RANK is a homotrimeric transmembrane protein receptor expressed in osteoclasts, osteoclast precursors, mammary glands, and some cancer cells (Xing and Boyce 139-146). RANKL-RANK binding activates intracellular signaling cascades in mesenchymal precursors and osteoclasts to induce osteoclast differentiation and osteoclastogenesis respectively (Nakashima et al. 2006). OPG is a soluble glycoprotein that is secreted by osteoblasts, bone marrow stromal cells, and other mesenchymal derived cells (McCarthy et al. 2009). OPG acts as a decoy/antagonist receptor for RANKL, binding the ligand without the creation of a cellular response thus lowering RANK-RANKL binding concentrations (Heymann et al. 2007).

RANKL/RANK/OPG interaction creates an important control system for bone modeling and remodeling via the control of osteoclastogenesis. Most signaling molecules mediate bone modeling/remodeling using this system to some extent (Matsuo and Irie 2008). Increased RANKL expression, especially by osteoblasts is important in initiated bone absorption and the modeling/remodeling process. On the converse, increases in OPG secretion and concentration inhibit osteoclast differentiation and osteoclastogenesis. Also, this system has major interactions with the immune system, particularly T-cells. T-cells express RANKL (and interferons for counter-regulation) and are believed to facilitate remodeling during both pathological and non-pathological responses to a given location (Choi and Arron 2000).

Several major diseases are attributed to problems involving these signaling pathways and their mechanisms. For example inducing an extreme number of osteoclasts or osteoblasts could possibly result in an abundant loss of bone structure (osteoporosis) or the secretion of an excess of extracellular matrix (osteopetrosis). Also, improper signaling could also cause mutations in the bone cells themselves creating cancerous cells. The carefully regulation of bone tissue is needed to ensure whole-body homeostasis and local force bearing structures.

Control of Chondrocyte & Cartilage Formation in Bone

Several intrinsic and extrinsic molecules regulate the growth and proliferation of chondrocytes. These signals are vital to the proper development of chondrocytes in the process of endochondral ossification, serving as safety mechanisms to avoid uncontrollable growth. Intrinsic molecules involved include transcriptional factors with the possible involvement of MAP kinases. Extrinsic factors present are peri-articular cartilage, subchondral bone marrow, skeletal muscle, and endocrine hormones (Liu and Deng 2005).

Major transcription factors involved in intrinsic control of the proliferation and differentiation of chondrocytes include the protein core binding factor a1 (cbfa1) and gene locus sex determining region Y, box-9 (Sox9). Cbfa1 is expressed highly in mature chondrocytes in the hypertrophic region and induces matrix calcification and elevates alkaline phosphatase synthesis in the cell (Komori et al. 2001). On the other hand, Sox9 expression is vital throughout the chondrocyte's entire life cycle. This transcription factor is responsible for the differentiation of stem cells to chondrocytes, and for chondrocytes maturation (Behringer et al. 2000). Sox9 is regulated at various stages of the chondrocyte life-cycle using a variety of signals at each of these stages. It has also been proposed that Sox9 can be controlled with physical interactions with β-catenin (Yuko, de Crombrugghe and Haruhiko 2005).

Extrinsic controls of chondrocyte differentiation and proliferation derive mainly from radial tissues. The positioning of a chondrocyte distal or proximal to either the peri-articular cartilage or opposite bone tissue has an integral role in the type of signaling present. Stem cells condensed in the peri-articular cartilage produce parathyroid hormone-related peptide (PTHrP), which signal the inhibition of chondrocyte maturity and hypertrophy (Weir et al. 1996). As a chondrocyte is forced proximal from this layer as new cells emerge/differentiate, PTHrP concentrations decrease resulting in the removal of this inhibition for maturity. Furthermore,

subchondral bone marrow located proximal to mature chondrocytes and trabecular bone tissue secrete chemokine stromal cell-derived factor 1 (SDF-1). These cytokines diffuse across bone tissue and into the cartilage causing maturation and increased MMP secretion by chondrocytes (Chiu 2007). As these cells move proximal towards bone tissue the concentration of SDF-1 increases causing an increase in hypertrophic characteristics and increased extracellular cartilage matrix degradation via MMP's.

Other extrinsic controls of chondrocyte differentiation and proliferation include the protein Indian hedgehog (IHH), bone morphogenetic proteins (BMP's), and local skeletal muscle tissue. First, IHH proteins are synthesized and derived from pre-hypertrophic chondrocytes and exert a paracrine control over neighboring chondrocyte maturation. This creates a negative feedback control of PTHrP synthesis in peri-articular cartilage stem cells. When a lineage of pre-hypertrophic chondrocytes becomes abundant, IHH concentration is increased, inhibiting PTHrP, and thus removing the inhibition of chondrocyte maturity allowing the line to become hypertrophic (McMahon, Hammerschmidt, and St-Jacques 1999).

Bones morphogenetic proteins (BMP), found in all types of bone cells to facilitate bone growth, are also an important signal for the growth and maturation of chondrocytes. In particular BMP-6 has been shown to both induce stem cell differentiation (Liu and Deng 2005) and increase the synthesis of collage type-X fibers and alkaline phosphatase activity in hypertrophic chondrocytes (O'Keefe et al.1999). It has also been proposed that BMP-6 could also serve as an intermediate messenger between the signals of PTHrP and IHH (McMahon, Hammerschmidt, and St-Jacques 1999).

Local skeletal muscle or smooth muscle tissues are also thought to have a signaling pathway for chondrocytes. Mechanical stress on these tissues has been shown to increase DNA, collagen, and proteoglycans synthesis in proliferating chondrocytes (Kamiya et al. 2008). The exact signaling pathway from mechanical stress to increased chondrocyte activity is still currently being investigated.

Bone Tissue Damage & Disease

The architecture and components of bone tissue allow for bones to withstand forces experienced throughout their lifetime. Different bones experience a different magnitude of forces depending on the degree of mineralization, collagen fiber orientation, bone shape, and mass (Ammann 2009). However as with all matter, bone can only withstand a finite amount of force upon it. For example, as stress is applied to over time, microcracks begin to appear in bone tissue. This damage does not impact the overall integrity of the bone and is usually repaired through the recruitment of normal bone remodeling mechanisms (Kennedy et al. 2005).

However, when larger forces from impacts/larger stress act on the bone greater structural damage can occur in the tissue. Resulting fractures can occur in three different types: wedging, shearing, or tearing. A wedge occurs when tissue is forced apart in opposite directions along the line of fracture. Shearing damage arises when tissue around the fracture's axis is forced anti-parallel in opposite directions. Finally, tearing occurs when tissue around the fracture is twisted away from the original plane of damage (Martin, Burr, and Sharkey 1998).

The physics of a non-pathological fracture derive from three different types of force: fatigue, traumatic loading, and quasi-static loading. Fatigue occurs from a combination of repetitive, everyday, normal activities creating stress on the bone, and inadequate remodeling to replace subsequent damage. Traumatic loading forces are much larger in magnitude than normal loading, and often come from impact or blunt forces. Quasi-static loads are forces that are applied to the bone slowly. A good example of this type could include an individual with osteoporosis whom loses bone tissue mineralization over time, slowly increasing the load upon the lamellar or trabecular layered structures (Currey 2005).

Certain diseases can also cause complications and weakening of bone structure resulting in fracture. Pathological fractures occur when the structure of bone has been compromised by local or systemic disease, resulting in damage. These diseases can be divided into the following groups of classification: constitution anomalies, myelogenous and inflammatory bone diseases, primary and secondary bone tumors, and posttraumatic/postoperative disorders. To which of these groups a particular disease is classified is a highly debated subject among researchers (Wirbel and Mutschler 1997) (Scott and McKusick 1971).

Homeostatic Fracture Repair

When a fracture occurs, other tissues such as the periosteum and surrounding soft tissues are usually damaged. The resulting tissue deformation provokes bleeding to form a hematoma, and cellular injury causing the release of cytokines to induce inflammation (Mora 2006). When inflammation begins, platelets, macrophages, and other inflammatory cells enter the wound to remove infection and advance clotting. Also they secrete a combination of cytokines and growth factors to induce new tissue generation (Little et al. 2008).

As the hematoma clots to form a fibrous thrombus, the area is clear of degenerated cells and growth factors bind and recruit mesenchymal stem cells from either local stromal tissue or from the circulation. The specific growth factors involved include: platelet-derived growth factor (PDGF), transforming growth-factor (TGF), tumor necrosis factor alpha (TNFα), and bone morphogenetic proteins (BMP's). Specific cytokines involved are interleukins 1 and 6 (IL-1, IL-6) (Papathanassopoulos et al. 2009). After around three days after the fracture, an increased concentration of inflammatory growth factors and cytokines coupled with an increased concentration of recruited MSC induces these stem cells to proliferate and differentiate into chondrocytes (Papathanassopoulos et al. 2009). These cells then begin to produce and secrete cartilaginous extracellular matrix to replace the fibrous thrombus tissue (Mora 2006). Resulting hyaline cartilage then fills between fragments of damaged bone creating a biological splint to support the local bone tissue. (Papathanassopoulos et al. 2009).

Continuing the process of endochondral ossification, as the chondrocytes mature and enter hypertrophy, the cartilage begins to mineralize. This occurs when osteoblasts begin entering the soft callus to create primary woven bone tissue, starting the creation of the hard callus structure (Little et al. 2008). Eventually the soft callus is completely replaced with this primary bone tissue to complete the formation of the hard callus structure. Once this has completed, bone remodeling begins transforming the woven tissue into secondary, lamellar cortical or trabecular tissue. The hard callus begins this remodeling typically around the four weeks after the initial fracture and finishes around the sixteenth week after, depending on the size of the wound (Mora 2006). Upon completion of remodeling, the former fracture has been completely replaced with the appropriate bone tissue for that location and the structure becomes a part of the functional unit once again.

Synthetic Tissue Repair/Facilitation

Introduction

Bone tissue is subject to various diseases, developmental deficiencies, and traumatic damage. Many structural problems arising from these issues require large portions of bone tissue to be removed or added. The replacement of these voids sometimes is simply too great or too time consuming of a task for the body to undertake. For example, the loss of a large mass of cortical tissue, secondary tissue that takes time to develop forces an individual to spend an lengthy amount of time in recovery. This time spent without regular, natural forces upon the bone may also have negative effects on the structure of other areas of tissue.

To avoid these problems and return functionally in a timelier manner after these occurrences, physicians over the last century have applied various combinations of materials and methods to facilitate the body's natural healing process. The goal was/is to create a substitute that will integrate with normal bone tissue, maintain bone's functionality, and have the capability to undergo normal bone tissue modeling and remodeling processes. This included the original use of splints and progressed to theories and the eventual use of bone transplantations. For years bone grafts have been popular but complications and problems with supply still arose. This directed research to develop synthetic materials that both provide the healing capabilities of bone grafts while avoiding the risks (Fages et al. 1998).

Today, these applications are applied in vivo to create a base for future bone formation. Materials that serve as this base are known as osteoconductive materials. Other terms used with these synthetic materials for the facilitation of bone growth include osteointegrative, osteoinductive, and osteogenetic. Osteointegrative implies that the material binds and adheres well with local bone tissue, while osteoinductive infers the ability to actively recruit bone progenitor cells. Osteogenetic is reserved for materials that possess resident or inserted stem cells and generate bone cells directly.

The search for materials to assist in bone repair has occurred for thousands of years, with concrete progress made during the scientific revolution and acceleration of the last couple of centuries. Besides bone grafts, early 20^{th} century synthetic materials developed involved acrylic cements to aid in the repair of bone fractures. This practice involved to the use of plasters to fill voids left by disease in bone, and eventually to calcium based compounds. In the last several

decades, these compounds have been altered to avoid the hazardous effects originally encountered and are still in use today.

Modern medicine has brought about the improved use of bone grafts and additional synthetic compounds like tricalcium phosphate, bioactive glasses, and glass ionomers. These compounds have shown great promise in the areas of osteointegration and osteoconduction. Also, the development of synthetic hydroxyapatite, the resident calcium phosphate mineral of bone tissue provides further benefits in current orthopedic surgery. Composite materials involving various combinations of the materials mentioned are the most common types of implantations today. Also, metals are commonly used in joint and screw implants, choosen for the durability and lifetime of these materials.

As science continuous to evolve with new research, numerous more options are becoming available to aid in bone tissue repair. Future studies in stem-cell research and gene-therapy could ultimately provide therapeutic treatments to individuals in need. The increasing interaction between biomedical sciences, engineering, physics, and mathematics will continue to produce biomechanical products that will provide many future implantation or applications in orthopedics.

Bone Grafting Introduction

Bone grafting involves the implantation of extrinsic bone tissue to a wound with the goal to achieve integration and new bone growth. Tissue used in bone graft can be cortical, trabecular, or a combination of the two. The idea of using bone tissue from a different individual (or even species) or different location in the body is not a new method in medicine. Grafting has been mentioned in early Greek Mythology, documented in cases as early as 1668 A.D., and gained tremendous popularity during the Second World War (Blitch and Ricotta 1996).

Bone grafts are obtained by removing tissue from a donor site, transplanting, and then embedding that tissue into a wound. Surgical operations gain access to the targeted site and remove the desired tissue from a donor. Low-speed power drills are used for the removal of the mineralized bone tissue, which is accomplished by drilling the donor tissue into strips, in an effort to resist donor site morbidity (Brawley and Simpson 2006). Sterilization procedures are commonly used to remove superfluous proteins, other cells and tissue, or possible pathogens, especially in allogenious bone grafts (Stevenson 1999).

In normal conditions, the insertion of the graft to wounded/damaged bone tissue creates a scaffold for the formation of new tissue. This scaffold minimizes the need for fibrous connective tissue formation, and provides a mass for eventual remodeling. After implantation, the area of the wound undergoes the normal tissue injury response, with inflammation and vascular invasion. Soon, the graft begins to adhere to bone tissue around and produce osteoinductive growth factors to recruit mesenchymal stem cells for osteoblast differentiation for bone formation. The graft fuses with resident bone tissue with normal fracture repair mechanisms; a soft callus is formed, mineralized, and then modeled to woven bone tissue. Eventually the graft becomes remodeling to secondary trabecular/cortical bone tissue and becomes an analogous component of the bone (Konttinen 1998).

Autogenious Bone Grafting

In a modern clinical setting, two different types of bone grafts are used: autografting and allografting. Autografting involves tissue transferred from one area to another in the same individual. This type of grafting begins with harvesting bone tissue from a donor location, commonly from the lower appendage or hip bones depending on the desired type of tissue. Autograft types include trabecular, lamellar cortical and woven cortical tissue (Stevenson 1999). Trabecular tissue's most common donor site is the iliac crest of the illium while cortical tissue is usually obtained from the ribs, tibia, or fibula (Jäger et al. 2005).

Trabecular tissue usually has the highest osteogenic and osteoinductive capacities compared to other tissue grafts, due to its rich vascularization and abundance of bone marrow (Konttinen 1998). These properties increase the chances for successful bone repair and remodeling, thus increasing the chances for successful tissue union and wound repair. However, with a less organized tissue structure compared to lamellar tissue, trabecular bone grafts do not provide the structural supported that may be needed at certain locations (Stevenson 1999). Also, the quantity of available trabecular tissue compared to other types is low without increasing the potential for donor site morbidity (Finkemeier 2002). Damage to the trabecular tissue surrounding the donor site can easily occur due to the interconnectivity of the local vascularity.

Lamellar cortical autografts provide significantly more structural support in a wound compared to trabecular tissue. The organized lamellar cortical structure allows for larger force bearing and transduction. The low vascularity and thickness of this tissue resists the onset of

bone remodeling, allowing it to act as a simple support/splint. In large or unique shaped wounds this tissue type provides immediate support to the surrounding bone and help to return partial functionality (Stevenson 1999). Also, nonvascularized cortical autografts can be used in conjunction with other types of grafts to provide structure and protection for their osteoinductive and osteoconductive processes (Finkemeier 2002).

Woven cortical bone tissue is the third type of bone graft used in autografts, and provides benefits intermediate to the previous two types. The tissue's architecture lacks the structure of lamellar, but has collagen fibers ordered in a much less random fashion than in woven trabecular tissue. This property allows the graft to provide greater structural support over woven trabecular tissue, while providing more vascularity and thus more osteoinductive capabilities than lamellar cortical tissue. The intermediate attributes also allow the graft to adhere and interact to a variety of bone tissue that may be found in the voided area of application (Stevenson 1999).

Benefits of autografts in bone or any other tissue are numerous. The consistency of the biological constituents between the donor and replaced tissue is a good starting point. Similar macromolecules between the two allows for better material adhesion and compatibility. Also, with the same antigen presentation adverse immune response or immuno-rejection to the graft is avoided. Finally, with material transferred within the same individual, the risk for infection is lowered due to the avoided use of separate individual donor bone (Finkemeier 2002).

While benefits of autografts are numerous, and considered the top choice for filling bone voids, complications and negative consequences may also arise. In particular, removal of tissue for the graft from the donor site increases the possibility for a complication. Since intrinsic tissue is being extracted, patients sometimes experience pain, infections, or fractures at the donor site. Also, the local functional structure of bone may become damaged from surgery, causing further complications and deficiencies. Other minor problems possible may occur due to the immobilization of the patients, such as blood clots. (Konttinen 1998).

Allogenious Bone Grafting

The second type of bone grafting, allografting, uses bone tissue from a different individual for implantation to a patient. The availability of allograft tissue from donation banks, patient relatives, and cadavers provides a more abundant volume of material for bone tissue repair than in autografting. Allograft tissue is removed from donors via procedures simular to

the removal of tissue in autografting. Prospective donors, as with any other type of tissue donation, undergo strict screening with guidelines set by groups such as the American Association of Tissue Banks (AATB), the American Red Cross, and the United States Food and Drug Administration (FDA). Once they have passed these standards they are approved for tissue donation (Lavernia et al. 2004).

After removal from a donor, allografts undergo extensive laboratory sterilization. While irradiation is the most common sterilization used, other methods include autoclaving or freezing drying the extracted bone. Freezing or freeze-drying the bone tissue destroys most of the resident cells and removes the antigenicity of the tissue (Nasser, Vandevord, and Wooley 2006). A combination of these processes allows for different types of bone tissue to be processed efficiently. In the end, different individual bone tissue allografts can be produced: demineralized bone matrix (DBM), morselized, and cortical whole-bone segment transplant (Finkemeier 2002).

DBM, created from any type of bone tissue, is removed from a donor in sterile conditions and then crushed or pulverized to particulates in a laboratory. The resulting bone tissue particles are then washed with an acid (usually HCl) in order to chemically induce demineralization and destroy resident cells. The acid is then washed from the particles using sterile water, ethanol, and ethyl ether, leaving a DBM product which is freeze-dried and stored in a certified tissue bank (Kostopoulos et al. 2003). The resulting powder substance is then combined with carriers such as gelatin or glycerol to prepare for application in the form of a putty or paste.

When a recipient is found, the DBM is then applied either directly or in a mixture with synthetic materials. With no living cells present this material is not osteoinductive, but various remaining proteins do provide minor osteoconductive properties. The particulate composition of the graft allows for easy vascular invasion to begin the repair process. However, the absence of any organized macro-structure and the complete absence of mineralization create a graft that cannot provide any structural support until after full local remodeling and integration (Finkemeier 2002).

The second form of bone allografting is prepared in the form of morselized bone tissue. Morselized allografts begin with any type of bone tissue, removed from a donor patient in sterile conditions and transferred to a laboratory for processing. Upon arrival at the laboratory, the donor bone tissue is crushed or ground into millimeter sized “chips” (much larger in size compared to DBM particles) and then preserved via freeze-drying. Freeze-drying kills most of

the resident cells removing antigenicity. Insertion of morselized bone "chips" into a recipient patient allows for regular vascular in-growth, initiating the inflammation and repair processes. However, lacking living cells means this type of graft is also only osteoconductive. However, since the chips still have intact collagen fiber structure and are still mineralized this allograft can lend some structural support to the targeted area. Versus DBM morselized allografting lacks the ability to allow for quick vascularization for repair, but provides better structural support and scaffolding for the targeted area (Stevenson 1999).

The third and final type of allografting includes cortical whole-bone transfer from a donor to a recipient patient. Most commonly obtained from cadavers, whole segments of bone are removed and then either freeze-dried or deeply frozen. When a prospective recipient is found the segment is transferred whole, trimmed to fit the targeted area for implantation, or trimmed for use as a structural strut for the application of other materials. The intact bone tissue contains both osteoinductive and osteoconductive properties, but undergoes slow vascular invasion, repair, and eventual integration due to the structure of cortical tissue. Cortical whole-bone allografts are common choices for patients whom have large voids in bone tissue or have voids in areas that require immediate structural support for proper function (Finkemeier 2002).

The large availability of bone tissue allografts are the signal largest beneficial reason this method is a popular choice for surgeons and their patients today. Rigorous donor selection and screening greatly lower the risk of any pathological or genetic problems in tissue donation. Also, compared to autografts, surgical procedures are shorter for individual patients. With allografts there are no secondary sites of operation, hence the duration of hospital stays have been shown to be much shorter. Also, there is no risk for a recipient to have tissue donor location problems that allografts have. All of these benefits point towards lower costs, from the larger supply of material, shorter operative and recovery time periods. Thus, allogenious bone grafts create a more cost-efficient orthopedic application that still has many of the same benefits as an autograft (Konttinen 1998).

Complications are possible in the application of allografts: such as secondary infection(s) from the recipient surgery and/or the donor patient experiencing problems with the tissue harvesting. There is also the risk of infectious disease transfer and/or immunological rejection no matter how intensely or carefully the tissue is processed. Also, while good screening and freeze-drying remove a large portion of antigenicity from the tissue, grafting is still the insertion

foreign material to the body. Rejection of the graft is the single largest negative consequence of allografting (Nasser, Vandevord, and Wooley 2006).

Synthetics: Acrylic Cements & Derivatives

As alternative materials and procedures to bone grafts were explored, the widespread use of bone cements arose during the middle of the 20th century. In the 1930's and 1940's the companies of Degussa and Kulzer began to research and develop self-curing cements with the intended use for orthopedic application. In 1958 Sir John Charnley successfully inserted a prosthetic into the head of a femur using self-curing cement. Since this time, the application of cements in bone tissue healing has been used regularly by surgeons across the world. Application of bone cement is commonly used to facilitate the union of prosthetics to bone; during both fixation of artificial joints and anchoring of implants (Gopp, Kuehn, and Ege 2005). Bone cements are osteointegrative to some extent, but are neither osteoinductive, osteoconductive, nor osteogenetic.

Original bone cements were a mixture of poly methylacrylate (PMMA) powder, liquid methylmethacrylate (MMA), and a heat sensitive initiator: creating the class of acrylic bone cements. Acrylic bone cements are prepared in the operating room by mixing a provided powder with a separate liquid in a method called the two component system. The powder is usually a mixture of PMMA or MMA copolymers, a curing initiator (dibenzoyl perioxide), antibiotics, and a radiopacifier for visibility in radiographs. The liquid usually contains MMA monomer and a small concentration of a curing activator. When two are mixed the polymer powder absorbs the liquid monomer creating a semi-solid/liquid substance, which is then applied to the desired targeted location using a paste or injection. Next, the initiator from the powder and the activator from the liquid interact to form free radicals. These free radicals facilitate the bonding of monomers to each other forming a solid substance. Remaining radicals and then are absorbed into the double bonds that are created or combine with each other. Hardening of the cement occurs between 6 to 10 minutes after initial mixing, depending on the exact composition (Gopp, Kuehn, and Ege 2005).

Immediately after application, bone cements provide great mechanical support for the surround bone tissue while lacking the invasiveness of grafting. When supporting prosthetic and joint implants this provides immediate stability and adhesion to the bone tissue. However the

incompatibility of the cement substrate with local tissue and the energy released from the curing process can cause substantial damage to surrounding tissue. Immediately the immune response begins to counter the damaged tissue and foreign material. This can result in bone cement implantation syndrome: a disease state from the toxicity of the cement eventually leading to cardiac problems/arrest (Burlingame 2009).

More commonly, damage caused by the cement substrate and curing heat results in the development of a fibrous connective tissue layer between the cement and the surrounding bone tissue. This tissue development isolates the cement from surrounding bone tissue and thus prevents the substrates integration into the bone as a whole. Without complete integration, the area is subjected to future complications, especially from the failure to disperse normal loading forces across the cement-scar tissue-bone structural deficiency (Collía et al. 2007).

Many of these negative consequences occur primarily with the acrylic bone cements. A combination of other types of mixtures developed, various toxicity reducers, and antiseptic substances have helped to reduce these complications. Other types of bone cements available today include calcium phosphate cements and silicon-containing cements (Bauer 2007). Calcium-phosphate cements provide osteoconductivity, due to their similarity to inherit mineral quality of bone tissue. The most common type includes a dicalcium phosphate dehydrate mixture with calcium oxide and liquid-phase sodium phosphate buffer (Alt et al. 2004). On occasion silicon is added to facilitate the cement's osteointegrative ability and biodegradability. The addition of silicon to this mixture encompasses the third class of cements: silicates (Chang and Huan 2009). Avoiding free radical curing (with subseqent heat production) and the replacement of toxic substances allow for this class of cements to avoid the major problems of acrylic cements. Changes in composition reduce the potential for fibrous scarring and even may eventually become integrated and replaced by secondary bone tissue (Alt et al. 2004).

Synthetics: Calcium Sulfate Ceramics

Another type of synthetic scaffolding developed to facilitate healing includes ceramic calcium sulfate materials. The original use of this material before its medical application provided it the name of the plaster of Paris. This plaster was and still is common used for adhesion needs, such as between bricks, for flooring, and so on. Medically, the first documented use for fracture treatment by Arabs in the 10th century who used this material as a splint to

immobilize whole bones after fracture. This use would continue to the development of bandages for fractures up until the 19th Century. In 1892 a German by the name of Dr. Dreesman successfully used calcium sulfate to treat tuberculosis osteomyelitis in vivo. Today calcium sulfate is used as a bone void filler particularly in small voids and in joint and hip replacements (Moore, Graves, and Bain 2001).

Originally, calcium sulfate was synthesized from calcium sulfate dehydrate (commonly known as gypsum) which is heated and oxygenated. This forms an anhydrous salt which is then hydrated to form a semi-solid paste, needing excessive heat or ignition for curing (Broughton 1939). Today, calcium sulfate is created using a mixture of calcium sulfate hemihydrate and hydroxypropylmethylcellulose (or derivative) that only requires mixing with water. The semi-solid product formed can either be injected or applied as a paste to the targeted site for implantation. The material hardens via polymerization through the hydration of the calcium sulfate hemi-hydrate and formation of bonds with hydroxypropylmethylcellulose, a reaction that is completed within minutes of mixing (Richelsoph et al. 2004).

Upon administration, calcium sulfate pastes solidify and bind to local tissue within minutes. Insertion forms a resorbable scaffold that is easily invaded by vascular processes to begin the repair process (Blaha 1998). The circulatory system brings mesenchymal stem cells to calcium sulfate which bind, differentiate to bone cells, and begin absorbing the material. After absorbtion, soft and hard callus formation remodeling begins as usual. This involves the complete absorption of calcium sulfate by osteoclasts and the formation of new extracellular matrix by osteoblasts. The calcium ions released from the material provide a partial supply of minerals for osteocytes to facilitate tissue mineralization. With calcium sulfate the formation of new bone tissue is expedited allowing the void to fill at a much quicker rate than what could occur naturally (Peters et al. 2006).

Benefits of calcium sulfate include immediate adhesion and good compressive strength. This makes these pastes great for the insertion of other scaffolds or artificial joints to facilitate the integration of these materials. Also, the ability to set quickly provides immediate mechanical stability increasing the return of normal bone functioning. Furthermore, as a synthetic material it lacks antigenicity, and with close composition to natural substances it is non-toxic in most modern applications.

Negatives with calcium sulfate use include the speed at which the material cures and the need for a dry environment for curing. Since the material cures so fast, application must occur quickly putting strain on the surgery completion time by applying a time frame to the operation. The need for a dry environment also creates a special problem, with bodies that are inherently full of fluids. This sometimes has the tendency to liquefy calcium sulfate possibly allowing the implant to shift or the loss of mechanical stability. Finally, other questions in sulfate and sulfate derivative compound toxicity also are possible, given the difference between these compounds and that of resident bone tissue (Peters et al. 2006).

Synthetics: Tricalcium Phosphate Ceramics

Tricalcium phosphate (TCP) effects on bone formation were reported as early as 1920, when it was given in the form injections to damaged bone tissue. TCP are still used today but in different applications: in the form of solid blocks or more frequently in granular form (Moore, Graves, and Bain 2001). These materials provide good osteointegration, osteoconduction, but lack osteoinductive and osteogenic properties. Often they are also mixed with other cements, glasses, or DMB, and usually antibiotics to avoid infection. TCP mainly are applied with the goal of creating new trabecular tissue, although porosity can be altered to reflect and stimulate cortical tissue formation (Vaccaro 2002).

To create TCP, the most common method recently has been the calcium-phosphate emulsion method. In this method alpha tricalcium phosphate is mixed with tricalcium phosphate and added to polyethoxylated castor oil consisting of polyacrylic acid and sodium hydrogen phosphate. This solution was then stirred until solid precipitation, cooled, poured into molds, and incubated for hardening. The solid is then cleaned with petroleum ether, dried, and sintered for purification. The resulting material porosity can be controlled by varying the concentration of the polyethoxylated castor oil, which forms droplets that create the pours. The final product is then ready for application, but can molded/drilled to desired shape or can grounded and pressed into pellets which are eventually mixed with agar (Müller et al. 2005).

Solid blocks of TCP are usually applied in combination with other grafting or synthetic materials to enhance compatibility, absorption, and osteoinductivity. Upon implantation, the blocks show little adhesion, but the porosity allows for quick fluid uptake and ability for vascularization. Typical cellular responses occur immediately with fused phagocytes

investigating the new material and inflammatory mechanisms responding to local tissue damage. Bone formation occurs when stem cells transverse through the pores, bind to the material, and differentiate to create bone cells for remodeling. Calcium and phosphate ions released from the material serve as a local mineral reserve for subsequent mineralization. Over several months (depending on size) after application, the block is absorbed via dissolution and fragmentation, and remodeled into resident bone tissue (Mirzadeh et al. 2008).

Granular TCP pellets are also frequently applied in combination with other materials. This type obviously has an increase in potential for fluid uptake and vascularization but does not provide the structural support blocks do. However, granular TCP can be absorbed and remodeled to resident bone tissue faster and more efficiently. After application, vascular in-growth occurs with the recruitment of stem cells for the differentiation of bone cells during remodeling. Some inflammation does occur from local tissue damage, stimulating stem cell differentiation and the formation of minor fibrous scar tissue prior to resident bone tissue formation (Burgess et al. 2004).

TCP materials are beneficial for implantation to bone tissue mainly for their compatibility and absorbability by the body. Since the material composition closely reflects that of resident tissue, it is easily absorbed and lacks toxicity. Also the lack of immunogenicity avoids the classic immuno-rejection problems, and synthetic composition avoids disease transfer and other related complications (Moore, Graves, and Bain 2001). Structurally, TCP blocks provide some structural support to the area and are porous enough to allow for bone cell differentiation, remodeling, absorption, and ultimate bone tissue formation. Granular TCP avoids problems of the block form's tendency to shift with dense application. While the granular does not provide the structure of block form, it does allow for expedited vascularization and resorption (Takeshita et al. 2006).

TCP material applications run into potential problems in several different areas. First, less bone volume is formed versus the volume of TCP absorbed, creating the possibility of material shrinkage and scar tissue formation (Moore, Graves, and Bain 2001). Next, while blocks provide some structural support, they are brittle compared to resident tissue and other synthetics, making their use in large volume needs very inefficient (Müller et al. 2005). TCP blocks poorly bond with existing local tissue and thus can shift in positioning inside the wound. Furthermore, research studies have shown that in some TCP implants, constituents that are

absorbed have shown up in local lymph nodes signaling the potential for complications in other organ systems (Peters and Reif 2004). Overall, these problems and the lack of osteoinductivity and osteogenicity are usually avoided by using TCP implants in composition with other synthetic or graft materials.

Synthetics: Bioactive Glasses & Glass Ionomers

Observing the success of silicon-based materials adhesion and integration to existing bone tissue, several other types of compounds known as bioactive glasses and glass ionomers were developed in the 1970's. Bioactive glasses are solid, non-porous materials consisting of sodium oxide, calcium oxide, phosphorus pentoxide, and silicon dioxide. These ingredients can be varied in concentrations to yield the bioactive glass completely absorbable to completely in-absorbable (Moore, Graves, and Bain 2001). These properties create a scaffolding material that is osteointegrative, osteoconductive, slightly osteoinductive, but not osteogenic.

Bioactive glasses are prepared by either by melting/mixing or sol-gel processing. In the melting/mixing procedure the regent ingredients are melted at high temperatures, mixed, sieved, poured through graphite mold, and then sintered (Mao et al. 2008). The sol-gel processing begins with the hydrolysis of alkoxide precursors to create a sol or colloidal liquid with silicon and other molecules, allowing gel formation after 3 days at an ambient temperature, and then heat dried for crystallization (Polak, Gentleman, and Jones 2007). Bioactive glasses are usually applied in a solid shape developed from molding, since the inherent structure resists drilling and shaping. Other administration methods include via granules or as a coating for metal implants, both of which are formed/applied before the drying phase (Moore, Graves, and Bain 2001).

The crystalline structural, bonding, and alkaline properties of bioactive glass allow for this material to integrate and adhere extremely well with existing bone tissue. When the material is implanted it begins to immediately release cations to the surrounding local tissue. As this occurs, local hydrogen ions are absorbed by the alkaline molecules left behind in the material from cation release, resulting in the hydration of the glass surface. The resulting increase of basicity in the environment also induces the release of silicon, facilitating further material to local tissue bonding. Eventually calcium and phosphate ions migrate to the surface from both the surrounding tissue and interior of the glass, condensing the silicon bonds and forming new bonds. Eventually these bonds form an overall strong adhesion between the bioglass and local

tissue that rivals intrinsic bone tissue bonds. Depending on the composition the material is either completely absorbed by osteoclasts, remodeled into secondary bone tissue, or undergoes a limited amount of local fibrous tissue formation. The properties of bioglass and the speed of adhesion usually avoid major immunological responses outside of the typical inflammatory response (Polak, Gentleman, and Jones 2007).

Around the same time of bioactive glass development, cements using similar properties were developed known as glass ionomers. This class of material was originally developed for dental use where cement was required to bind in a moist environment. Glass ionomers contain calcium, aluminum, and flurosilicate glass powder mixed with polycarboxylic acid or phosphoric acid (Moore, Graves, and Bain 2001). The successful use in dentistry for over 20 years without a significant adverse reaction prompted the development of orthopedic-grade material (Hurrell-Gillingham, Brook, and Hatton 2006). Analogous to bioactive glasses, glass ionomers are osteointegrative, osteoconductive, slightly (but still not clearly) osteoinductive, but not osteogenic.

Glass ionomers are prepared by mixing the powder mixture with the acidic liquid prior to administration. The acid causes cation release from the powder to produce a phosphate hydrogel matrix which holds other various cations in place in the developing crystalline structure. The mixture is then administered via injection or a paste to the targeted site. After administration, the acid-base reaction continues until the material completely solidifies. Binding with local bone tissue begins with the release of ions from the implanted glass ionomer. In particular, fluoride anion is released into the local environment and stimulates alkaline phosphatase activity of local cells. This increases the basicity of the environment, facilitating mineralization and bonding between the glass ionomers and local bone tissue. Eventually, depending on the ionic makeup of the material, the glass ionomer cement maybe completely reabsorbed or undergoes local fibrous tissue formation (Pearson, Williams, and Billington 2006).

Bioactive glasses and glass ionomers provide numerous benefits for bone tissue union in wound healing. Both materials bond extremely well to local bone tissue versus acrylic and derivative cements, and are also susceptible to absorption and integration. Glass ionomer cements avoid curation problems that acrylic cements have, by using an acid-base catalyzed polymerization reaction instead of damaging exothermic free radical reactions. Both materials also provide immediate strength and stability for the local tissue (Polak, Gentleman, and Jones

2007) (Hurrell-Gillingham, Brook and Hatton 2006). Furthermore, the materials are not immunogenic, and may even regulate the release of cytokines in inflammatory response. Finally, the release of silicon during material and tissue bonding may provide a small osteoinductive capability that other cements lack, although this mechanism is still debated. (Day and Boccaccini 2005).

As with any synthetic material application to tissue, complications with bioactive glasses and glass ionomers do arise. When the compositions of these materials are altered to the point of in-absorbability, the risk of fibrous scarring arises. This increases the potential for non-union and future structural deficiencies in the tissue. Also, fused monocytes may sometimes recognize these materials as foreign bodies; generate immune responses that create swelling/discomfort for long periods of time. Furthermore, the metal ions found in some of these materials may be toxic to local cells, inhibit tissue growth/development, induce neurological complications, and possibly weaken the recipient's immune system (Polak, Gentleman, and Jones 2007) (Hurrell-Gillingham, Brook and Hatton 2006).

Synthetics: Hydroxyapatite

Hydroxyapatite, the resident calcium phosphate mineral in mineralized osseous tissue, began synthetic preparation in the 1970's. Currently, synthetic hydroxyapatite is available in ceramic or non-ceramic, solid or porous forms of blocks or granules. Ceramic hydroxyapatite are resistant to resorption in vivo, while non-ceramic is readily absorbed. Solid hydroxyapatite mostly resists resorption with vascular in-growth and provides great structural support, while porous has converse properties. Blocks of hydroxyapatite provide great structural support but are hard to shape, while granular hydroxyapatite is easy to apply in vivo, and is common used in joint and screw implantations. This allows for material that varies in osteoconductivity, provides great osteointegration, but lacks both osteoinductivity and osteogenicity without supplemental materials (Moore, Graves, and Bain 2001).

Synthetic hydroxyapatite is prepared from natural apatite minerals using precipitation or hydrolysis under non-acidic conditions with the titration of phosphoric acid. Ceramics are formed via sintering to form a denser product from the resulting condensed crystalline structure. Non-ceramics may be formed under high pressure or simple precipitation and will vary in density. Foaming agents like hydrogen peroxide or naphthalene can be added to the liquid phase

of either ceramic or non-ceramic to create porosity. Blocks of hydroxyapatite can be formed using molds to create the desired shape. Pellets are formed by crushing other hydroxyapatite product material, applying gelatin or agar, and shaping. Prepared material is then applied to a recipient commonly mixed with tricalcium phosphate, and often times other scaffolding materials (Park, 2009).

The type of hydroxyapatite implanted depends upon the recipients' need. Ceramic hydroxyapatite materials are applied in locations where great structural strength is needed immediately, but will resist resorption and remodeling for great lengths of time (Park, 2009). Non-ceramic hydroxyapatite may provide mechanical strength depending upon application type, and will readily become absorbed by local osteoclasts and remodel into resident bone tissue (Komlev, Barinov, and Orlovskii 2002). Porous material allows for easy vascular permeation throughout, allowing for bone cells to begin the remodeling process sooner. Granular hydroxyapatite creates the capability for even easier vascular invasion, and can be used where remodeling of resident tissue is needed quickly (Komlev et al. 2001). Blocks of hydroxyapatite serve mostly as a structural unit providing support and stability in larger sized voids (Moore, Graves, and Bain 2001). Regardless of the type of synthetic hydroxyapatite used all are biocompatible and will eventually, over a period of time, become absorbed and remodeled by bone cells.

The benefits of using synthetic hydroxyapatite as scaffolding in the artificial repair of bone defects are numerous. The most obvious is the lowered risk of complications from immune-rejection, toxicity, and non-compatibility with local tissue. Hydroxyapatite is the most abundant mineral found in bone tissue and using it as a component in scaffolding provides the body with an already synthesized source. When bone cells move to the modeling and remodeling processes involved in tissue damage, this mineral is easily regulated into its position amongst collagen fibers. The second main benefit is the degree of variability that allows this material's properties to be greatly varied from need to need. Changes in the preparation are all that are required to alter the composition of the product. This creates synthetic hydroxyapatite that can be used as a small void filler to be absorbed quickly, or as a dam to prevent in-growth of fibrous connective tissue where necessary. Third, the ability to mix this with other types of materials creates possible composites that increase the chances for proper bone healing (Park, 2009). Lastly, new research shows that the ability to administer drugs via mixing with carefully

chosen material properties would enable a steady release over a desired period of time into the circulatory system (Komlev, Barinov and Orlovskii 2002).

Consequences for the use of hydroxyapatite may not be numerous, but nonetheless are present. While hydroxyapatite may provide good compressive strength, they are notoriously brittle and weak under tension and shearing forces (Moore, Graves, and Bain 2001). Next, as with any implant comes the possibility that the material shifts after original insertion: possible causing local damage, pain, or negative tissue scarring. Finally, using the extremes of types available may introduce respective problems. For example having a material too porous could result in structural deficiencies, while having one too dense may resist resorption for long periods of time resulting in the buildup of scar tissue locally.

Synthetics: Aluminum Oxide, Other Metals, & Carbon Derivatives

In the early 20th century, aluminum oxides were developed and used as industrial cutting tools and insulators. As the 1970's came around, the beneficial application of this material to dentistry and orthopedics was researched and applied in vivo. Today this material is commonly used in the manufacture of medical implants (Park, 2009). The inherent strength in aluminum oxides make them ideal for orbital implants, joint linings, and other areas subjected to high mechanical forces and stress. However, aluminum in vivo does not exchange ions with resident bone tissue and therefore is not osteointegrative. Aluminum oxides also lack osteoconductivity, osteoinductivty, or osteogenicicity (Moore, Graves, and Bain 2001).

Medical grade aluminum oxide is obtained by calcination of aluminum hydroxide precipitated by aqueous aluminum nitrate and aqueous ammonia, and subsequent protracted grinding. This produces an aluminum oxide powder, which can be further pulverized to finer grade, or mixed with additives to enhance desired properties. The powder then can be shaped to a desired shape by applying pressure and heat, cooled, then sintered for purity (Lashneva, Kryuchkov and Sokhan 1998).

The prepared aluminum oxide implant or powder coating is then applied to a recipient under general surgical conditions. While the material does not integrate directly with osseous tissue, the hardness and lack of degradation provide strength and support for various splints, screws, and coatings for other material. Since aluminum oxides do not bind directly to the tissue they are usually used in the form of screws or in combination with other materials if placement is

long-term. If an aluminum oxide implants were to be used for filling voids in bone tissue or without other scaffolding materials, then the inflammatory response would develop fibrous scar tissue between the implant and local bone tissue. The development of this scar tissue could eventually undermine local bone structure and the original intended purpose of the implant (Park, 2009).

However, there are several benefits to the lack of osteointegration of aluminum oxides, in particularly in the lining of artificial joint implants. The long-term tribological properties are favorable for these applications. Aluminum surfaces facilitate the chemiabsorbtion of long-chain carboxylic acids and water via hydrogen bonding, allowing for a decrease in friction, wear volume, and surface roughness over time. These properties along with the tensile strength of aluminum oxides make them ideal candidates for areas that are susceptible to high stress, impacts, or mobility (Park, 2009).

Negative consequences include long-term wear of the metal, the aforementioned lack of integrative/conductive mechanisms with local osseous tissue and the inherent toxicity of aluminum. Physical wear over time occurs when the aluminum oxygen crystal lattices begin to degenerate and lose structure. Slowly cracks can begin to form, spreading from pre-existing flaws until larger scale deficiencies are created (Park, 2009). The last consequence of aluminum is its toxicity throughout the body. Release of aluminum ions can cause local dissolution of minerals from bone tissue endangering structural integrity, especially in woven bone tissue. Other consequences of free aluminum ions include renal failure, hemolysis, encephalopathy, others, and the destruction of various cellular membranes due to the formation of free radicals/oxidative stress (Becaria, Campbell and Bondy 2002).

Other materials either in current use for tissue engineering/scaffolding include zirconium oxides, titanates, carbon coatings, and diamond-like carbon coatings. Zirconium oxides provide strength to implants, specifically artificial joints, via the crystalline structure formed by zirconium and oxygen molecules. Zirconium oxide also directly binds with local bone tissue, resists inflammatory responses, and up-regulate enzymes associated with tissue growth. This substance can be commonly found lining titanium screws and in composition with other materials (Bignozzi et al. 2008).

Titanium, found in the same periodic group of zirconium has similar benefits in application to bone tissue. Titanates provide great structural support, have good tribological

properties and are able to osteointegrate to some degree with local bone tissue (as with zirconium). However, these metal alloys lack the required properties to increase local bone growth that zirconium does, and do not bind as well. Titaniums are currently the material of choice for screws and other metal implants, and also may be used as coatings in other composite implants (Brook and Freeman 2006).

Finally, recent research is investigating the use of carbon nano-tubes and diamond-like carbon coatings to enhance scaffolding materials and new bone tissue generation. The structure of nanotubes provides great support for local tissue and the bonding ability of carbon allows for ostointegration. Using carbon nanotubes in conjunction with other materials (usually metals) allows for the creation of stronger adhesion, thereby resisting local fibrous tissue generation (Sahithi et al. 2010). Nanocrystalline diamond coatings mimic the roughness of bone tissue, provide good strength, and are biocompatible. Bonding also occurs readily with local bone tissue creating great adhesion. In vitro research shows that this material could have inductive effect on osteoblast recruitment and proliferation, signaling possible osteogenic effects in vivo (Lopes et al. 2008). Current and/or potential uses for both carbon and diamond-like carbon coatings include in composition with other materials to facilitate adhesion or even to produce osteogenic effects and increase the rate of new osseous tissue formation.

Other/Future Methods for Bone Tissue Generation

Most of the materials developed for tissue engineering are only created with classical scaffolding characteristics: osteointegrative and osteoconductive. On the contrary, most lack the essential characteristics in inducing new bone tissue formation, osteoinduction, and mechanisms needed for the differentiation of new bone cells: osteogenicity. Recently several methods have been developed or are currently under research to provide the latter two characteristics in order to increase the efficiency of new tissue generation in a voided area.

Osteoinductive methods currently include extraction and purification of growth factors and recombinant protein synthesis. The knowledge of various factors controlling bone growth, modeling, and remodeling provide a good resource for choosing purifying certain molecules to facilitate bone healing. However, this process is not perfect and has more research to be completed. Osteogenic methods, on the other hand have shown immediate usefulness in recent

research. Examples include the use of stem cells, bone morphogenic proteins (BMPs), and gene therapy (Liu and Deng 2005).

In recent decades, the therapeutic use of stem cells has gained interest in its potential biomedical applications. Bone marrow provides the largest reserve for stem cells that eventually differentiate into red blood cells and/or bone cells. The first type of marrow: red is found in both flat bones and trabecular bone tissue at the ends of long bones. This type contains mostly hemapoietic stem cells and endothelial stem cells, providing the differentiation of blood cells. The second type of marrow, yellow, begins to slowly develop in woven tissue medial and deep in the long bones. Yellow marrow contains a high percentage of fat and stromal tissue containing mesenchymal stem cells (Milwid and Parekkadan 2010).

While both hemapoietic stem cells also have some capability to differentiate into bone cells, mesenchymal cells and their primary role in this process have been singled out for orthopedic therapy. During bone tissue healing mesenchymal cells migrate to the voided area and differentiate into the required bone cells for repair (Liu and Deng 2005). Facilitating synthetic material/scaffolding with a combination of these cells would increase the efficiency of the bone healing process. This would add osteogenicity to materials that lack this property.

Currently mesenchymal cells are obtained by bone marrow transplantation, cultivating/growing, and introducing/reintroducing them to a recipient. In both methods, removal of bone marrow occurs using either an aspiration procedure, with a syringe, or whole bone removal and subsequent marrow isolation. Regardless of the extraction method, mesenchymal stem cells can be then applied either directly in vivo via injection or in vitro to scaffolding material which can then be implanted in vivo. This method shows clinical promise, although further research is needed in purification, sterilization, and antigenicity investigative avenues (Kraus and Kirkerhead 2006).

A second osteoinductive method under investigation for clinical implementation involves the use of bone morphogenic proteins (BMPs). BMP were originally discovered by Marshall Urist in 1965, who isolated a bone inductive extract from bone tissue and demonstrated its ability to induce new endochondral bone formation (Liu and Deng 2005). As described in the control of bone growth and formation section, these proteins play an integral role in the differentiation of mesenchymal stem cells into various bone cells and stimulate activity. Over 30 known types exist today and all (except or BMP-1) are a part of the TGF-β super family. BMP are simply

extracted from bone tissue, isolated, purified, and concentrated for clinical use. Their purchase is currently available from several various pharmaceutics companies (Nilesh et al. 2007) (Liu and Deng 2005).

In particular BMP-2 and BMP-7 have shown great clinical use. BMP-2 has been shown to promote the differentiation of osteoblasts from mesenchymal cells in vitro and in vivo, helping provide the cellular framework for bone tissue formation (Kim et al. 2009). BMP-7, also known as osteogenic protein-1 (OP-1) also recruits and induces mesenchymal stem cell differentiation to osteoblasts (Vock et al. 2006). BMPs can be combined with mesenchymal stem cell extracts in fibrin hydrogel to increase the interaction prior to application in vivo, or addition to scaffolding material in vitro. While current use of BMP have shown few side-effects, continued clinical use and biomedical research is required to understand all possible effects (Liu and Deng 2005).

Finally, the third method employed to bring osteoinductive and osteogenic properties to synthetic wound healing includes the trial use of gene therapy. This application uses either hemapoietic or mesenchymal stem cells and aims to provide them with genes encoding specific osteoinductive growth factors to enhance the healing response (Liu and Deng 2005). For success the proper gene must be isolated, applied to an appropriate delivery vector, and implanted upon appropriate scaffolding material. Various genes have been targeted for use, but genes encoding BMP along with vascular endothelial growth factors (VEGF) have gained particular interest in their ability to induce osteoblast differentiation and angiogenic capabilities respectively (Evans 18).

For transfer, cDNA vectors with adenoviruses have been chosen, although other carriers are currently under consideration. Whichever vector is chosen is then applied to either hemapoietic or mesenchymal stem cells in vitro, mixed/plated to scaffolding material and implanted in vivo. The transferred BMP genes then produce an autocrine effect upon the stem cells causing them to differentiate into osteoblasts. Also the transferred VEGF gene allows for the creation of cytokines by the stem cells that induces blood vessel growth and subsequent transport of more, local stem cells via circulation (Evans 2010).

Gene therapy however has yet to be fully initiated in clinical trials (or even in vivo), citing the concerns of immunogenesis, fate of adenovirus, long-term complications, and the general risks/issues associated with the method itself (Liu and Deng 2005). Further progress in

the overall field of gene therapy is a prerequisite for the potential use of gene alteration to aid in wound healing in vivo.

Conclusion

The relationships between mineralized extracellular matrix and cells throughout bone tissue create a dynamic, efficient material that provides support to the body. The amazing capabilities of osteoblasts, osteocytes, and osteoclasts to: secrete, adapt, and repair masses of extracellular matrix much larger then themselves, is key to these properties. Together, the mixture of both organic fibers and inorganic minerals allows for bone to provide tensile strength and still remain susceptible to interaction with cells. Careful signal coupling amongst bone cells and between other systems in the body regulates these interactions.

On occasion damage occurs to bone, either from structural deficiencies or outside forces, however the body has mechanisms in place to efficiently repair and heal damage to bone tissue. Small damage, known as microcracks, occur frequently are simply remodeling and replaced by bone cells without any major damage occurring to bones as a whole. Larger scale damage, usually as the result of disease or trauma, are also eventually repaired and healed by the body, but using larger scale and more time consuming mechanisms.

Either way, the body is well suited on its own to deal with these problems. In the course of everyday remodeling or larger fracture repair, complications rarely occur. Millions of years of evolution has developed a system that has an extremely high success rate of proper healing versus any complications. Rarely, if ever, is outside intervention necessary to assist the body with healing. However, sometimes certain situations from trauma or disease sometimes require external or internal intervention. When damage to bone tissue is either large in magnitude or unique in character, to the extent that healing cannot occur normally, several therapeutic procedures and materials are available for the facilitation of healing.

For thousands of years humans have used tools, in the form of wood and metals to stabilize and immobilize bones after damage/breaks. This provided the tissue with a stable environment for which the body could effectively repair the damaged tissue. Later, plaster casts were eventually developed to provide both a lighter weight and cheaper material to protect the healing process, still in use today. While these materials provide external support, a need still existed for in vivo tissue healing facilitation. Implanted materials were needed to provide

scaffolding that would support local tissue and facilitate new tissue generation in unique or large scale voids from damage. Also, an increase in procedures that replaced joints created a need to develop materials that bridge body and mechanical devices. This led to procedures including bone grafts, the insertion of various materials, and synthetic compounds.

Bone grafts are considered the "golden standard" in the facilitation of bone tissue healing. Using tissue removed from a donor or another location on an individual patient provides bio-material that matches the composition of local tissue around the damage. However, the availability of bone tissue for grafting and the fear of damage local tissue provided the need for synthetics. Allogenious grafts from donors and/or cadavers dilute this problem, but also is material found in limited supply. Also, the antigenicity and risks from surgical operations involved in the transplantation of the graft can produce further complications for individuals involved. This has led to the development of synthetic, engineered materials in attempt to avoid the problems with bone grafting.

The production of bone cements, ceramics, glasses, and synthetic hydroxyapatite provide materials with varying properties, while avoiding the complications of bone grafts. Early forms of these materials, especially acrylic cements, has complications in integration with existing tissue and toxicity to the body. For example, some early cements applied in vivo would fail to bond with local tissue, causing the development of fibrous scar tissue between the two. Other issues faced also included the failed capability of absorption, leaving a material that never integrates fully with bone tissue.

Recent advances in composition and development of biocompatible materials have produced alternative compositions that widely avoid these issues. New knowledge of bone tissue composition and advances in material engineering has produced materials that bond well with local tissue and can be absorbed over time. This implantation provides both scaffolding material that facilitates new tissue growth while providing physical support for existing tissue. Today, synthetic products are commonly used in combination with each other, taking advantage of the different chemical and physical properties each provides. These materials are even compatible with existing artificial joints, helping connect "man and machine". The availability of composite materials to surgeons today allows them to provide customized care for the facilitation of bone healing on a need by need basis.

Future research will undoubtedly discover new materials and enhance existing materials and procedures. However, if we can prevent or limit tissue damage before these implants are needed, then the negative consequences of surgery and administration of foreign material to our body can be avoided. Recent discoveries in bone biology show that knowledge of the system's functioning is still not fully understood. Advances in this area will eventually provide new insight into how this dynamic tissue functions, and how this knowledge can be used to develop therapies to strengthen and expedite the repair processes. Finding methods to combat tissue disease and damage at the biological source, without surgical intervention, will provide a more effective tool in maintaining functional bone structure and healing. Thus, preventative medicine is the key to the future of bone health.

Ultimately, the facilitation of bone health and proper bone healing rests with the actions of individuals themselves. While sometimes diseases and accidents are outside of one's control, many basic complications can be avoided with positive lifestyle choices by individuals themselves. Living a healthy, active lifestyle is crucial in the prevention of weakened bone tissue and avoids the need for major intervention in healing. Exercise builds muscle to support bone, and provides a constant loading forces to stimulate the remodeling and re-strengthening of the tissue. Consuming a healthy diet provides the body with the necessary compounds to maintain homeostasis without the need for the body to constantly tap bone's mineral reserve and weaken bone tissue. Finally, avoiding risky activities will avoid the chances of experiencing traumatic forces that could result in large scale bone tissue damage.

Bone tissue is a dynamic interaction of solid tensile, material and cells that constantly adapts to environmental stimuli, providing individuals with support and strength throughout the body. Discipline, care, and education in an individual’s own health are key to ensuring that these properties functional. However, if disease or trauma creates the need for healing facilitation, the combination of advances in biomedical research, advances in biomedical material engineering, and orthopedic surgery create many options for individuals to return to normal health. With proper bone health, we all have the capability to live long, active, independent, and prosperous lives.

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