UNIVERSITI PUTRA MALAYSIA

ROLE OF PLASMA MEMBRANE TRANSPORTERS (N+/H+ AND HCO3-) IN MEDIATING MAMMALIAN LONGITUDINAL BONE GROWTH AND FRACTURE HEALING

ABUBAKAR ADAMU ABDUL

FPV 2016 26

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By

ABUBAKAR ADAMU ABDUL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

November 2016

DEDICATION

This project is dedicated to my family and humanity at large, especially my parents for their unconditional love and infinite supports given to me in my life journey.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

ROLE OF PLASMA MEMBRANE TRANSPORTERS (N^+/H^+ AND HCO_3–) IN MEDIATING MAMMALIAN LONGITUDINAL BONE GROWTH AND FRACTURE HEALING

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November 2016

Chairman : Loqman Mohamad Yusof, PhD
Faculty : Veterinary Medicine

Mammalian long bone growth and secondary bone healing occur by means of endochondral ossification, which involves tightly controlled cellular differentiation of chondrocytes at the epiphyseal region and callus formation at the fracture site. Although the cellular mechanism of chondrocyte differentiation that regulates long bone growth and fracture healing is still poorly understood, plasma membrane transporters were thought to have the mediating roles. This study aimed to investigate the roles of Na^+/H^+ antiporter (NHE1) and HCO_3^- anion exchanger 2 (AE2) in linear bone growth and secondary fracture healing. The specific objectives were: (1) To study postnatal *ex vivo* rat model for longitudinal bone growth investigations. (2) To investigate the role of Na^+/H^+ (NHE1) and HCO_3^- (AE2) exchange across membrane of chondrocytes in mammalian longitudinal bone growth. (3) To investigate the effect of recombinant human growth hormone (rhGH) on the localisation of NHE1 and AE2 membrane transporters on long bone growth in rat. (4) To establish metatarsal fracture model in rats for *in vivo* investigation of secondary bone healing. (5) To investigate the role of NHE1 (Na^+/H^+) and AE2 (HCO_3^-) membrane proteins during secondary bone healing in rat.

Firstly, an experiment was undertaken to determine the suitable age to study bone growth using rat pups *ex vivo* model. The result showed direct bone sectioning for histology was possible across all age groups in metatarsal bone rudiments and in 7-13 day-old pups tibia. However, tibial sectioning was relatively difficult in 14 and 15 day-old rats. Significant differences in tibia and metatarsal growth plate (GP) length was observed among different age groups at different incubation periods (P<0.05). Significant differences of chondrocyte densities in the GP of tibia and metatarsal were recorded before and after 72 hrs incubation. *Ex vivo* longitudinal growth of tibia and metatarsal bone of rats at age of 7-15 day-old was possible under conducive

physiological condition and maximum growth rate was observed in tibia of 10 day-old rats (P10).

Postnatal (P10) metatarsal and tibial bones were cultured for 48 hrs *ex vivo* in the presence of plasma membrane inhibitors of 5-ethylisopropyl amiloride (EIPA) and 4,4'-diisothiocyano-2,2"-stilbenedisulfonic acid (DIDS) for NHE1 and AE2 respectively. The study revealed bone growth suppression by approximately 11% at concentration of 444 µM and 250 µM of EIPA and DIDS respectively. The two inhibitors had no significant effect on the total GP length but significantly affect the total GP and HCZ chondrocytes densities. There was no significant difference between NHE1 and AE2 localisation and fluorescence signaling across GP length. The remarkable suppression of bone growth along with the inhibition of chondrocytes proliferation at the entire GP and HCZ by EIPA and DIDS was an indication that the plasma membrane proteins (NHE1 and AE2) have potential role in bone growth through regulation of chondrocytes density.

In order to determine whether there is effect of bone growth inhibition by EIPA and DIDS on bone growth stimulation under the influence of growth hormone (GH), P10 rat metatarsal and tibia were cultured for 48 hrs in the presence of GH in combination with EIPA, DIDS or the vehicle, DMSO (control). Results showed bone cultured in DMSO recorded steady growth similar as treatment with additional GH. In the presence of GH fluorescence labeling of NHE1 and AE2 membrane proteins along GP was enhanced along with increased in the longitudinal bone growth. However, the combination of GH with EIPA or DIDS suppressed longitudinal bone growth, total GP length, GP chondrocytes density and localisation of NHE1 and AE2 along the GP. The fluorescence labeling of NHE1 and AE2 were also significantly inhibited in EIPA+GH or DIDS+GH treatments.

The present study also established a reproducible transverse mid shaft 3rd metatarsal fracture model for laboratory investigations. The model produced a fracture at the shaft of metatarsal bones that was 100 % transverse, 73% located at mid shaft with minimal fracture angulations based on radiographic evidence (0.48 ± 0.09^{O} at anterior posterior view; 0.78 ± 0.17^{O} at lateral view). There was minimal soft tissue injury, no infection or delayed bone union observed. Varying degree of weight bearing lameness was initially observed but subsequently absent at day six onwards post-surgery. Callus index was observed to peak in week 2 and 3 (2.02 ± 0.1 and 1.99 ± 0.13, respectively) but declined to 1.10 ± 0.04 in week 7 during consolidation period. There was no significant difference between the histological and radiographic healing scores at week 7 post-surgery.

Chondrocytes in fracture callus could be detected as early as first week of bone healing, which peaked after 3 weeks and subsequently declined and ceased at week 6. NHE1 and AE2 localisation was recorded throughout the period of healing but peak signaling was recorded in the first 4 weeks of healing and then significantly declined from week 5 onwards to week 7. The NHE1 localisation was significantly higher than that of AE2 during the healing

period but there was no significant difference of mean localisation signaling score between NHE1 and AE2.

In conclusion, EIPA and DIDS have significantly inhibited longitudinal bone growth, HCZ length and total GP chondrocyte density. Expression of NHE1 and AE2 was affected by the inhibition of EIPA and DIDS in the presence of GH. The transporters were also found to be present in the fracture site at significant high level throughout the first 4 weeks of fracture healing period. This result suggested the possible role of NHE1 and AE2 in longitudinal bone growth as well secondary fracture healing.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PERANAN PENGANGKUT MEMBRAN PLASMA (NA^+/H^+ DAN HCO_3^-) DALAM MEMBANTU PERTUMBUHAN LONGITUDINAL DAN PENYEMBUHAN TULANG BAGI MAMALIA

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Pertumbuhan tulang panjang dan penyembuhan tulang sekunder bagi mamalia berlaku melalui proses pembentukan tulang endokondral yang melibatkan pembezaan sel kondrosit yang dikawal rapi di kawasan fiseal dan pembentukan kalus pada tempat kecederaan. Walaupun mekanisma pembezaan sel kondrosit yang mengawalatur pertumbuhan dan penyembuhan kecederaan tulang panjang masih kurang difahami, pengangkut membran plasma dikatakaan dapat memainkan peranan sebagai pengantara. Kajian ini dibuat untuk menyiasat peranan Na^+/H^+ antiporter1 (NHE1) dan HCO_3^- penukar anion 2 (AE2) dalam pertumbuhan linear tulang dan penyembuhan kecederaan sekunder. Objektif khusus adalah: (1) Untuk menjalankan kajian *ex vivo* pada model tikus selepas beranak untuk mengkaji pertumbuhan tulang membujur. (2) Untuk mengkaji peranan pertukaran Na+/H+ ((NHE1) dan HCO_3^- (AE2) merentasi membran kondrosit dalam pertumbuhan tulang membujur mamalia. (3) Untuk mengkaji kesan hormon pertumbuhan manusia rekombinan (rhGH) di lokasi NHE1 dan AE2 membran pengangkutan sepanjang pertumbuhan tulang dalam tikus. (4) Untuk mewujudkan model kecederaan pada metatarsal tikus dalam kajian *in vivo* bagi penyembuhan tulang kedua dan (5) Mengkaji peranan NHE1 (Na+/H+) dan AE2 (HCO_3^-) protein membran semasa penyembuhan tulang kedua tikus.

Eksperimen pertama telah dijalankan untuk menentukan umur yang sesuai bagi mengkaji pertumbuhan tulang menggunakan model *ex vivo* anak tikus. Hasil kajian menunjukkan hirisan tulang untuk histologi boleh dibuat pada semua peringkat umur bagi tulang metatarsus dan pada tulang tibia anak tikus yang berusia 7-13 hari. Namun begitu, hirisan tulang tibia agak sukar diperolehi daripada anak tikus yang berusia 14 dan 15 hari. Perbezaan yang signifikan pada panjang pola pertumbuhan (GP) tibia dan metatarsus diperhatikan secara histologi dalam peringkat umur yang berbeza pada

tempoh inkubasi yang berlainan (P<0.05). Tiada perbezaan signifikan bagi ketumpatan kondrosit dalam GP pada tulang tibia dan metatarsus direkodkan sebelum dan selepas 72 jam inkubasi. Pertumbuhan longitudinal tulang tibia dan metatarsus tikus yang berumur 7-15 hari secara *ex vivo* boleh terjadi di bawah keadaan fisiologi yang sesuai dan kadar tumbesaran yang maksimum didapati pada tibia tikus yang berusia 10 hari (P10).

Dalam kajian yang berikutnya, tulang metatarsus dan tibia P10 telah dikulturkan selama 48 jam secara *ex vivo* dengan penambahan *5-ethylisopropyl amoliride (EIPA)* dan *4,4'-diisothiocyano-2,2"-stilbenedisulfonic acid (DIDS)* masing-masing sebagai perencat membran plasma bagi NHE1 dan AE2. Keputusan menunjukkan perencatan pertumbuhan tulang dalam anggaran 11% masing-masing pada kepekatan 444 µM EIPA dan 250 µM DIDS. Kedua-dua perencat tidak mempunyai kesan yang signifikan pada panjang keseluruhan GP tetapi secara signifikan dapat mengurangkan panjang saiz zon kondrosit hipertrofi (HCZ) dan kepadatan keseluruhan kondrosit GP (P<0.05). Walau bagaimanapun, kepadatan HCZ secara relatifnya kekal malar. Tindakbalas setempat NHE1 dan pengisyaratan berpendarfluor seluruh panjang GP adalah lebih tinggi daripada AE2. Perencatan luar biasa pertumbuhan tulang longitudinal yang setara dengan proliferasi panjang zon HCZ oleh EIPA dan DIDS bersama dengan kepadatan kondrosit yang secara relatifnya malar dalam zon adalah petunjuk yang baik bahawa fenomena pengawal atur isipadu mungkin terlibat dalam proses pemanjangan pertumbuhan tulang di mana NHE1 dan AE2 mungkin mempunyai peranan mengawalselia proses di peringkat sel.

Dalam usaha untuk menentukan sekiranya EIPA dan DIDS mempunyai sebarang kesan perencatan pada pertumbuhan tulang melalui proliferasi rangsangan pertumbuhan tulang oleh hormon pertumbuhan (GH), metatarsus dan tibia tikus P10 telah dikulturkan selama 48 jam dengan kehadiran GH bersama kombinasi EIPA, DIDS atau sarana dan DMSO (kawalan). Keputusan menunjukkan tulang yang dikulturkan dalam DMSO merekodkan pertumbuhan yang stabil manakala penambahan GH dalam media kultur menyebabkan rangsangan langsung pada pertumbuhan tulang longitudinal, panjang keseluruhan GP dan kepadatan keseluruhan kondrosit GP. Dengan kehadiran GH, pelabelan pendarfluor membran protein bagi NHE1 dan AE2 sepanjang GP telah dipertingkatkan selari dengan pertambahan pertumbuhan tulang secara longitudinal. Walau bagaimanapun, kombinasi GH dengan EIPA atau DIDS telah merencatkan pertumbuhan tulang secara longitudinal, panjang keseluruhan GP, kepadatan kondrosit GP serta tindak balas setempat NHE1 dan AE2 sepanjang GP. Pelabelan pendarfluor bagi NHE1 dan penukar anion AE2 didapati dihalang secara signifikan dalam kumpulan yang diberi EIPA+GH atau DIDS+GH.

Kajian ini juga telah menunjukkan keratan rentas model retakan bahagian pertengahan metatarsus ketiga menghasilkan keputusan yang boleh diulang semula bagi mengkaji peranan pengangkut membran dalam penyembuhan tulang sekunder. Model kecederaan menghasilkan retakan pada bahagian tengah tulang metatarsus yang melintang pada kadar 100% di mana 73%

didapati pada pertengahan tulang dengan penyudutan yang minimal apabila dianalisa menggunakan radiografi (0.48 ± 0.09° pada pandangan anterior posterior; 0.78 ± 0.17° pada pandangan lateral). Kecederaan tisu lembut yang minimal, ketiadaan jangkitan atau penyambungan tulang yang tertunda telah direkodkan. Namun begitu, pada permulaannya, pelbagai tahap ketempangan galas berat diperhatikan yang kemudiannya beransur hilang mulai hari ke-6 selepas pembedahan. Indeks kalus dilihat mencapai tahap paling tinggi pada minggu ke-2 and ke-3 (masing-masing pada 2.02 ± 0.1 dan 1.99 ± 0.13) tetapi berkurangan menjadi 1.10 ± 0.04 pada minggu ke-7 semasa peringkat pengukuhan. Skor penyembuhan yang diperhatikan secara histologi dan radiografi masing-masing adalah 3.5 ± 0.13 dan 3.75 ± 0.25 (berbanding dengan skor maksimum penyembuhan iaitu 4) pada minggu ke-7 selepas pembedahan. Kajian ke atas proliferasi kondrosit (CP) menggunakan antibodi monoclonal mencit terhadap antigen proliferasi sel nuklear (PCNA) menunjukkan kondrosit dalam kalus retakan boleh dikesan seawal minggu pertama penyembuhan tulang, mencapai tahap tertinggi selepas minggu ke-3 dan kemudiannya berkurangan dan berhenti pada minggu ke-6. Terdapat perbezaan yang signifikan (P<0.05) pada CP pada selang masa penyembuhan yang berlainan. Tindakbalas setempat NHE1 didapati lebih tinggi secara signifikan dari tindak balas setempat AE2 semasa tempoh penyembuhan tetapi tiada perbezaan purata skor pengisyaratan setempat yang signifikan antara NHE1 dan AE2. Kesimpulannya, EIPA dan DIDS secara signifikannya boleh menghalang pertumbuhan tulang longitudinal, panjang HCZ dan kepadatan keseluruhan kondrosit GP walaupun dengan kehadiran GH. Ekspresi NHE1 dan AE2 juga dipengaruhi oleh perencatan disebabkan oleh EIPA dan DIDS walaupun dengan kehadiran GH. Kedua-dua pengangkut juga ditemui hadir dalam tempat kecederaan pada ahap yang tinggi secara signifikan sepanjang empat minggu tempoh penyembuhan kecederaan. Keputusan ini menunjukkan potensi peranan NHE1 dan AE2 dalam pertumbuhan tulang longitudinal dan juga penyembuhan tulang sekunder.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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µg	Micro gram
µL,	Micro liter
µM	Micro molar
ABC	Avidin biotin complex
AE2	Anion exchanger 2
ANOVA	Analysis of variance
AP	Anterior posterior view
AQP	Aquaporin membrane protein
ARF	Animal resource facility
BMP	Bone morphogenic protein
BSA	Bovine serum albumin
Caγ	Calcium gamma channel protein
CC	Capacitative coupling
cm	Centimeter
CMF	Combine magnetic field
CO_2	Carbon dioxide
DAB	3,3'-Diaminobenzidine
DBM	Demineralized bone matrix
DC	Direct current stimulation
DIDS	DIDS (4,4-diiodothiocyano-2,2-stilbenedisulphonate)
DMSO	Dimethyl sulphate
ECM	Extra cellular matrix
EGP	Epiphyseal growth plate
EHCZ	Early hypertrophic chondrocytes zone

EIPA	(5-(N-ethyl-N-isoprophyl) amiloride)
FCS	Fetal calf serum
FGF	Fibrolast growth factor
g	Gram
GA	Glutaraldehyde
GH	Growth hormone
GP	Growth plate
GPC	Growth plate chondrocytes
H&E	Hematoxyline and eosin staining
H_2O_2	Hydrogen peroxide
HCO_3^-	Hydrogen bicarbonate ion
HCZ	Hypertrophic chondrocytes zone
HMA	Hexamethylene amiloride
hr	Hour
IACUC	Institutional animal care and used committee
IF	Immuno-fluorescence
IGF	Insulin-like growth factor
IgG	Immunoglobuline G
IHC	Immunohistochemestry
IHH	Indian hedgehog
IMM	Induced micro motion
IP	Immuno Peroxidase
IU	International unit
K_{ATP}	Potassium ATP channel protein
kg	Kilogram

$KHCO_3$	Potassium bicarbonate
LHCZ	Late hypertrophic chondrocytes zone
LIUS	Low intensity ultrsound
M	Molar
MIBA	5-(N-methyl-N-isobutyl) amiloride
mL	Milliliter
mL^{-1}	Per milliliter
mM	Milimolar
mm	Millimeter
mV	Milivolt
N^+/H^+	Sodium and Hydrogen ions
Naγ	Sodium gamma channel protein
NBCs	Sodium couple bicarbonate cotransporter
NCX	Sodium calcium exchanger
ng	Nano gram
nm	nano meter
NHE1	Sodium hydrogen exchanger 1
NKCC1	Sodium potassium chloride co transporter 1
NMDA-R	N-methyl D-aspartate receptor
PAP	Peroxidase anti-peroxidase
PBS	Phosphate buffer saline
PEMF	Pulse electromagnetic field
PCZ	Proliferative chondrocytes zone
PDGF	Platelet derived growth factor
pH	Potential for hydrogen ion

PMCA	Plasma membrane Ca^{2+} ATPase
PTH	Parathyroid hormone
rhGH	Recombinant human growth hormone
RHT	Ruthenium hexamine trichloride
RMP	Resting membrane potential
RVD	Regulatory volume decrease
RVI	Regulatory volume increase
SEM	Standard error of means
SLC	Solute carrier protein
SPSS	Statistic for social sciences
TGF	Transforming growth factor
TGP	Total growth plate
TRPV	Transient receptor potential cation channel
UV	Ultraviolet light
v/v	Volume by volume
VEGF	Vesicular endothelial growth factor
w/v	Weight by volume
wtn	Want signaling pathways
α-MEM	Alpha modified essential media

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Bone is one of the organs of the musculoskeletal system which provide shape, physical support, to the vital organs of the body and facilitate the body movement (Clarke, 2008; Oryan *et al*., 2015). It is rigid, hard, and it can be easily regenerated and repaired (Taichman, 2005). It help store the marrow, serve as a storage facility for calcium, phosphorus, growth factors and cytokines, it is also involves in acid-base balance and endocrine regulation of energy metabolism (Peavey, 2003; Sanchez, 2006, Lakhkar *et al*., 2013).

Bone growth and development occur in two phases during embryonic development when bone tissues starts to develop and the second phase during postnatal life (Summerlee, 2001). The growth and development of the cortical component of the bone is usually taking place either through intramembranous or endochrondral ossification (O'Connor *et al*., 2010). Intramembranous ossification involves formation of flat bones, particularly those of craniofacial origin, which includes bones of the skull, facial bones, and the clavicle (Anderson and Shapiro, 2010; Long and Ornitz, 2013). In this process bone is develop directly from the mesenchymal stem cells precursors (Clendenning and Mortlock, 2012). The mesenchymal cells form an aggregate, which are then invaded by blood vessels and subsequently differente and proliferate into mature osteoblasts (Colnot *et al*., 2004; Yoshida *et al*., 2008; McBratney-Owen *et al*., 2008). The mature osteoblasts will then secrete osteoid, which directly laid down the foundation which will become flat bones (DeLise *et al*., 2000; Dallas and Bonewal, 2011) as shown in Figure 1.1.

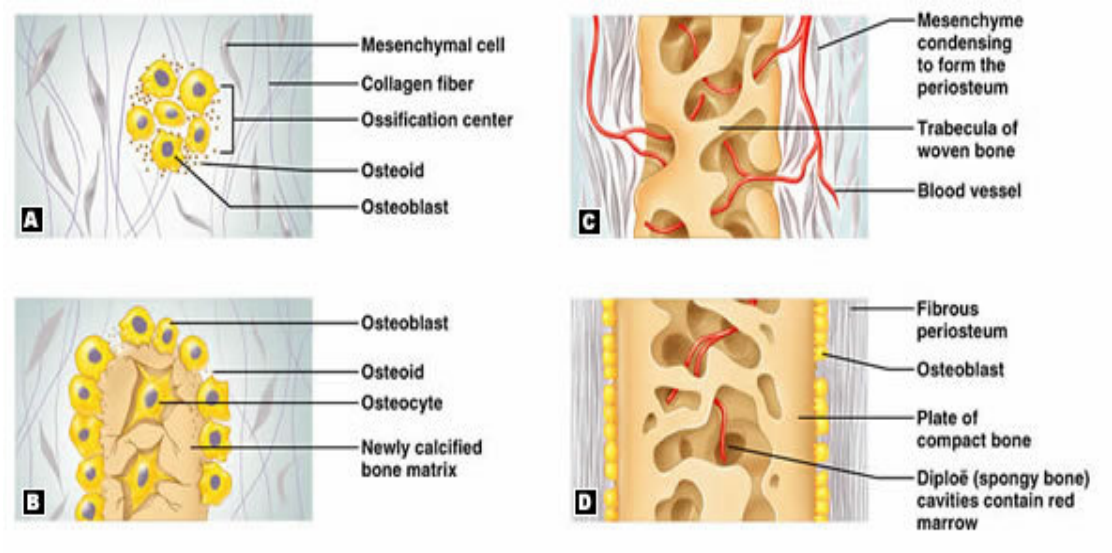

Figure 1.1 : Schematic diagram of sequential stages of intramembraneous ossification. Adapted from Pearson Education Inc., Cambridge, 2006

Endochondral ossification is relatively more complex than intramembraneous process of bone growth and development; it utilizes an intermediary stage of cartilage formation for the development of long bones. Secondary bone healing also occurred through the endochondral process of bone formation (Kronenberg, 2003; Mackie *et al*., 2011). Endochondral ossification is initiated by embryonic mesenchymal cells that aggregate to form a cartilaginous template which resemble the size and shape of the developing bone (DeLise *et al*., 2000; Staines, *et al*., 2013). The chondrocytes within the cartilage template then differentiate to form proliferative chondrocytes which subsequently undergoes morphological change into hypertrophic chondrocytes (Nilsson and Baron, 2004; Dowthwaite *et al*., 2004), the hypertrophic chondrocytes then undergoes programmed cell death before becoming vascularized, mineralized and invaded by osteogenic cells, and other precursor cells as shown in Figure 1.2 (Ornitz and Maries, 2002; Emons *et al*., 2009; Long *et al*., 2014).

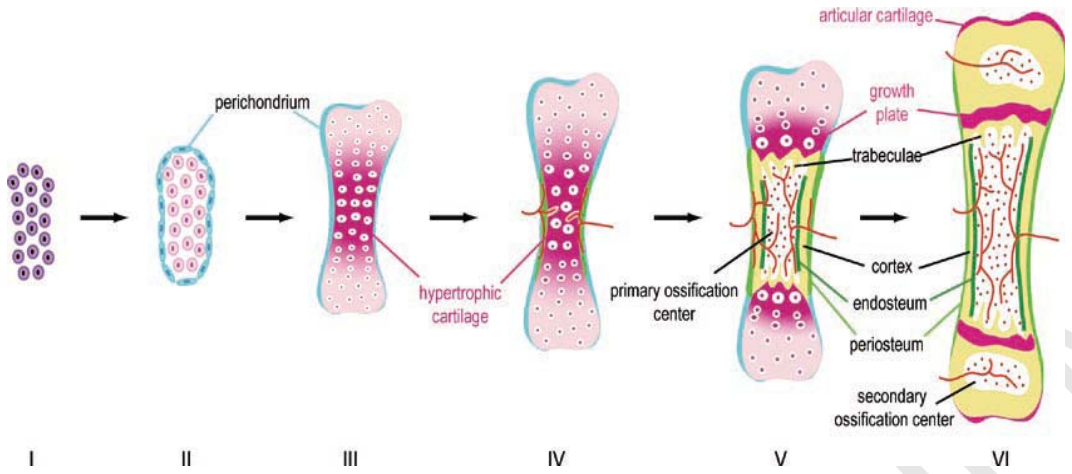

Figure 1.2 : Stages of endochondral ossification
(i) mesenchymal condensations, (ii) cartilage template, (iii) chondrocyte hypertrophy, (iv) vascular invasion, (v) formation of the primary ossification center, and (vi) formation of the secondary ossification center, and (vi) formation of the secondary ossification center and longitudinal growth. Adapted from Pearson Education Inc. Cambridge, 2006

There are numerous signaling factors that influenced the longitudinal bone growth these includes endocrine, paracrine and transcription factors. The signaling pathways play key roles in proliferation and differentiation of numerous cells that that take part during bone development (Yang and Karsenty, 2002; O'Connor *et al*., 2010; Staines *et al*., 2013). The endocrine factors are hormones that are involves in the regulation of bone growth development which includes; growth hormone, thyroid hormone, parathyroid hormone, calcitoriol, parathyroid hormone, estrogen, testosterone and calcitonin (Woods *et al*., 2007; Bobick and Kulyk, 2008; Beier and Loeser, 2010; Wit and Camacho-Habner, 2011). There are several paracrine signaling pathways that were reported to have played critical roles during bone growth. These includes; wtn signaling, Indian hedge hog [IHH], transforming growth factor [TGF-β], bone morpho genic protein [BMP], vesicular endothelial growth factor [VEGF], fibroblast growth factor [FGF] and platelet derived growth factor [PDGF] (Cooper *et al*., 2013; Abad *et al*., 2002; Joeng and long, 2014; Cho *et al*., 2012; Zhang *et al*., 2012; James, 2013; Maxhimer *et al*., 2015; Rahma *et al*., 2015). Bone growth is also regulated by many genes, majority of which are transcription factors which play important roles in the expression of genes that contribute significantly to bone growth, the most important transcription factors includes Sox9, Runx2, Dlx5, Twist1, c-Fos/AP-1, NF-κB, MITF, and NFATc1 (Nakashima and Crombrugghe, 2003; Guenou *et al*., 2005; Komori, 2006; Zhou *et al*, 2006; Yavropoulou and Yovos, 2008).

There are numerous ion channels and transporter proteins that were hypothesized to have potential role in in the course of bone growth and development according to Barret-Jolley *et al*., (2010); Lewis *et al*., (2011a). The functional activities of these ion channels and transporters were reported to have been taking place in the articular chondrocytes of the bone, which subsequently affect longitudinal bone growth (Lewis *et al*., 2013). The ion channels and transporter proteins so far reported to have potential role in long bone growth includes; NKCC (Na^+, K^+ and Cl^-), NMDA-R (Ca^+ and Na^+), AQP ($3H_2O$), ENaC (Na^+), Naγ (Na^+), Caγ (Ca^{2+}), K_{ATP} (K^+), TRPV4 (Ca^+ and Gd^+), TRPV6 (Gd^{2+}), TRPV5 (econazole Gd^{3+}), KZP (K^+), CIC (Cl^-), PMCA (Ca^{+2}), NCX ($3Na^+$), NHE (Na^+ and H^+), AE (HCO_3^- and Cl^-), VGSC (Na^+) and VGCC (Ca^+) (Bush *et al*., 2010; Butterworth, 2010; Lewis *et al*., 2011b; Yool and Campbell, 2012; Loqman *et al*., 2013; Karakas and Furakanu, 2014).

The ion channels and membrane proteins directly affect the functional activities of the chondrocytes physiology through resting membrane potential (RMP), which subsequently bring about regulation of chondrocytes volume. The chondrocytes volume regulation occurred when the RMP is at a steady state according to Hoffman *et al*., (2009); Lewis *et al*., (2011a). The physiological functions of the entire ion channels can be pharmacologically antagonised using their specific or non specific chemical antagonist. However, the mechanisms of action of most of the ion channels transporters that bring about modulation of chondrocytes physiology in bone elongation is either unknown or still controversial (Barrett-Jolley *et al*., 2010).

Currently there is no known report of their potential roles in fracture healing, however it can be hypothesized that, they may have similar potential role to play in the course of fracture healing, because longitudinal bone growth and fracture healing both occure through the process of endochondoral ossification.

Currently, studies were conducted on three ion channels (NKCC1, NHE1 and AE2) on their roles in chondrocytes hypertrophy with possible effect on longitudinal bone growth (Bush *et al*., 2010; loqman *et al*., 2013). The results of these studies have revealed that NKCC1, NHE1 and AE2, membrane transporter proteins act on hypertrophic chondrocytes via regulatory volume mechanism which bring about bone growth. The three membrane proteins transporters belong to solute carrier (SLC) classes, with different families and functional classes (Landowski *et al*., 2012; Saier Jr *et al*., 2013). Generally, the SLC transporter protein comprises genes that are responsible for passive transport, coupled ion transport and ion exchange (Hoffman *et al*., 2009).

Previous studies that were conducted to investigate role of plasma membrane transporters in long bone growth utilized metatarsal bone model for *ex vivo* bone growth. Although metatarsal bone is a typical long bone and has advantage of having multiple number bone rudiments that can be use to

have adequate sample size for statistical analysis but most at time is difficult to carefully harvest them with intact articular cartilage becaue of the smaller size of the bone. Therefore, there is need to explore other long bones like tibia that could be use as an *ex vivo* bone growth model in order to overcome the challage associated to the use of metatarsal bone.

Previous *ex vivo* bone growth model also used embryonic and post natal rat pup at day 7 (P7) bones. The choice of the embryonic and post natal P7 bone was that, both were proven not to be mineralized; hence there is no need to decalcify them in order to maintain their *in situ* morphological structure when histologically sectioned as reported by Loqman *et al*., (2010). However, there is no known documented age limit at which post natal bone is completely mineralized.

Previous investigators have hypothesized that, the cyclical process of endochondral bone formation occurred within a period of 24 hrs cycle, however, Chagin *et al*., (2010) and Okubo *et al*., (2013) reported that, the complete cyclical period of endochondral ossification can take place in more than 24 hrs period. This also warrant further investigation, hence our bone culture growth period was extended to 72 hrs with every 24 hrs change of media and taking bone length parameters.

1.2 Problems Statement

1. Bone growth disorders and complications of fracture healing lead to great economic and social impact to the public health, individual life quality and losses in livestock industry. Therefore, experimental investigations involving role of plasma membrane transport in bone growth and fracture healing may help explore the mechanism and pathogenesis of certain musculosketetal conditions.
2. Growth plate chondrocytes (GPC) hypertrophy has been implicated to be main determinants of bone growth rate through endochondral ossification but the mechanism is not fully understood.

1.3 Justification of the Study

The outcome of the study is expected to confer better understanding of the fundamental cellular mechanism of cell differentiation and tissue growth under normal and pathologic condition with emphasis on the role of plasma cell membrane transporters proteins. The plasma membrane protein in future could be of clinical relevance in mediating the cellular healing process of secondary fracture bone healing. Previous *ex vivo* studies of long bone growth have established potential roles of NKCC1, NHE1 and AE2 plasma membrane proteins during long bone growth.

1.4 Research Hypothesis

Plasma membrane transporters (Na^+/H^+) and (HCO_3^-) may play role in chondrocytes differentiation process that can contribute positively to the rate of mammalian long bone growth and secondary fracture healing

1.5 Objectives

The main objective of the work is to investigate the role of specific plasma membrane transporters (Na^+/H^+) and (HCO_3^-) during long bone growth and secondary fracture healing

Specific objectives:

1. To study postnatal *ex vivo* rat model for longitudinal bone growth investigations
2. To investigate the role of Na^+/H^+ (NHE1) and HCO_3^- (AE2) exchange across membrane of chondrocytes in mammalian longitudinal bone growth
3. To investigate the effect of recombinant human growth hormone (rhGH) on the localisation of NHE1 and AE2 membrane transporters on long bone growth in rat
4. To establish metatarsal fracture model in rats for *in vivo* investigation of secondary bone healing
5. To investigate the role of NHE1 (Na^+/H^+) and AE2 (HCO_3^-) membrane proteins during secondary bone healing in rat

REFERENCES

Abad, V., Meyers, J. L., Weise, M., Gafni, R. I., Barnes, K. M., Nilsson, O., Bacher, J. D., & Baron, J., (2002). The role of the resting zone in growth plate chondrogenesis. *Endocrinology,* 143:1851-1857.

Abràmoff, M. D., Magalhães, P. J., & Ram, S. J., (2004). Image processing with imageJ. *Biophotonics International, 11*(7): 36-41. doi:10.1117/1.3589100

Adler, C. P., (2000). Bones and bone tissue: normal anatomy and histology. In C. P. Adler (Ed.), *Bone Diseases* (pp. 1-30):Springer

Ahmed, S. F., & Farquharson, C., (2010). The effect of GH and IGF1 on linear growth and skeletal development and their modulation by SOCS proteins. *Journal of Endocrinology*, *206*: 249-259. doi:10.1677/JOE-10-0045

Ahmed, Y. A., Tatarczuch, L., Pagel, C. N., Davies, H. M. S., Mirams, M., & Mackie, E. J., (2007). Physiological death of hypertrophic chondrocytes. *Osteoarthritis and Cartilage, 15*(5): 575-586. doi:10.1016/j.joca.2006.10.016

Ai-Aql, Z. S., Alagl, S., Graves, D. T., Gerstenfeld, L. C., & Einhorn, T., (2008). Molecular mechanisms controlling bone formation during fracture healing and distraction osteogenesis. *Journal of Dental Research*, *87*(2): 107-118. doi:10.1177/154405910808700215

Alemdaroğlu, K. B., Tiftikçi, U., Iltar, S., Aydoğan, N. H., Kara, T., Atlihan, D., & Ateşalp, S., (2009). Factors affecting the fracture healing in treatment of tibial shaft fractures with circular external fixator. *Injury*, *40*(11): 1151-6. doi:10.1016/j.injury.2008.12.011

Alka, K., & Casey, J. R., (2014). Bicarbonate transport in health and disease. *IUBMB Life*, *66*(9): 596-615. doi:10.1002/iub.1315

Alper, S. L., (2006). Molecular physiology of SLC4 anion exchangers. *Experimental Physiology, 91*(1): 153-161. doi:10.1113/expphysiol.2005.031765

Alvarez, J. S., Balbin, M., Santos, F. F., Ferrando, S., & Lopez, J. M., (2000): Different bone growth rates are associated with changes in the expression pattern of types II and X collagens and collagenase 3 in proximal growth plates of the rat tibia. *Journal of Bone and Mineral Research,* 15 (1): 82-96.

Alvarez, J., Horton, J., Sohn, P., & Serra, R., (2001). The perichondrium plays an important role in mediating the effects of TGF-beta1 on endochondral bone formation. *Developmental Dynamics,* 221(3): 311–321.

Amini, S., Veilleux, D., & Villemure, I., (2011). Three-dimensional in situ zonal morphology of viable growth plate chondrocytes: a confocal microscopy study. *Journal of Orthopaedic Research, 29*(5): 710–7. doi:10.1002/jor.21294

Amith, S. R., & Fliegel, L., (2013). Regulation of the Na/H Exchanger (NHE1) in breast cancer metastasis. *Cancer Research, 73*(4): 1259-1264. doi:10.1158/0008-5472.CAN-12-4031

Andersen, T. L., Abdelgawad, M. E., Kristensen, H. B., Hauge, E. M., Rolighed, L., & Bollerslev, J., (2013). Understanding coupling between bone resorption and formation: A rereversal cells the missing link? *American Journal of Pathology, 183*: 235-46.

Andersen, T. L., Sondergaard, T. E., Skorzynska, K. E., Dagnaes-Hansen, F., Plesner, T. L., Hauge, E. M., & Delaisse, J. M., (2009). A physical mechanism for coupling bone resorption and formation in adult human bone. *The American Journal of Pathology*, *174*(1): 239-247. doi:10.2353/ajpath.2009.080627

Anderson, H. C., & Shapiro, I. M., (2010). The Epiphyseal growth plate. In Bronner, F., Farach-Carson, M. C. and Roach, H. I. (Eds.), *Bone and Development, Topics in Bone Biology* (Vol. 6. pp. 39-64): Springer

Andreassen, T. T., & Oxlund, H., (2001). The effects of growth hormone on cortical and cancellous bone. *Journal of Musculoskeletal and Neuronal Interactions*, *2*(1): 49-58. doi:1108-7161

Aranda, V., Martínez, I., Melero, S., Lecanda, J., Banales, J. M., Prieto, J., & Medina, J. F., (2004). Shared apical sorting of anion exchanger isoforms AE2a, AE2b1, and AE2b2 in primary hepatocytes. *Biochemical and Biophysical Research Communications, 319*(3): 1040-1046. doi:10.1016/j.bbrc.2004.05.080

Arvidson, K., Abdallah, B. M., Applegate, L., Baldini, N., Cenni, E., Gomez-Barrena, E., & Finne-Wistrand, A., (2011). Bone regeneration and stem cells. *Journal of Cellular and Molecular Medicine, 15*(4): 718-746. doi:10.1111/j.1582-4934.2010.01224.x

Aubin, J. E Lian, J. B., & Stein, G. S., (2006). Bone formation: maturation and functional activities of osteoblast lineage cells. In Favus, M. J. (Ed.), *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism* (pp. 20-29.): American Society for Bone and Mineral Research

Aubin, J. E., & Heersche, J. N. M., (2003). Bone cell biology osteoblasts, osteocytes, and osteoclasts. *Pediatric Bone: Biology and Diseases*, 43-75. doi:10.1016/B978-012286551-0/50004-X

Aurégan, J. C., Coyle, R. M. , Danoff, J. R., Burky, R. E., Akelina, Y., & Rosenwasser, M. P., (2013).The rat model of femur fracture for bone and mineral research. *Bone Joint Research,* 2: 149-54.

Bab, I. A., & Sela, J. J., (2012). Cellular and molecular aspects of bone Repair In Sela, J. J., & Bab I. A., (Eds.), *Principles of Bone Regeneration* (pp. 11-41): Springer. DOI 10.1007/978-1-4614-2059-0_2.

Baiguera, S., Macchiarini, P., & Ribatti, D., (2012). Chorioallantoic Membrane for *In vivo* investigation of tissue-engineered construct biocompatibility. *Journal of Biomedical Material and Research B Applied Biomaterial,* 100: 1425-1434.

Bailón-Plaza, A., & van der Meulen, M. C. H., (2001). A mathematical framework to study the effects of growth factor Influences on fracture healing. *Journal of Theoretical Biology, 212*(2): 191–209. doi:10.1006/jtbi.2001.2372.

Baker, A. R., Hollingshead, P. G., Pitts-Meek, S., Hansen, S., Taylor, R., & Stewart, T. A., (1992). Osteoblast-specific expression of growth hormone stimulates bone growth in transgenic mice. *Molecular Cell Biology, 12*(12): 5541-5547.

Balke, M., Neumann, A., Kersting, C., Agelopoulos, K., Gebert, C., Gosheger, G. Buerger, H., & Hagedorn, M., (2010). Morphologic characterization of osteosarcoma growth on the chick chorioallantoic membrane. *BMC Research Notes,* 3: 58.

Ballock, R. T., & O'Keefe, R. J., (2003a). Physiology and pathophysiology of the growth plate. *Birth Defects Research Part C - Embryo Today: Reviews*, *69*(2), 123–143. doi:10.1002/bdrc.10014

Ballock, R. T., & O'Keefe, R. J., (2003b). The biology of the growth plate. *The Journal of Bone and Joint Surgery. American Volume, 85-A*(4): 715-726.

Barlt, R., & Frisch, B., (2009). Bone Biology. In R. Barlt (Ed.), *Osteoporosis Diagnosis Prevention and Therapy* (Vol. 2. pp. 7-27): Springer. DOI: 10.1007/ 978-3-540-79527-8.

Barneaud-Rocca, D., Etchebest, C., & Guizouarn, H., (2013). Structural model of the anion exchanger 1 (SLC4A1) and identification of transmembrane segments forming the transport site. *Journal of Biological Chemistry, 288*(37): 26372-26384. doi:10.1074/jbc.M113.465989

Baron, R., & Kneissel, M., (2013). WNT signaling in bone homeostasis and disease: from human mutations to treatments. *Nature Medicine, 19*(2): 179-192. doi:10.1038/nm.3074

Barrett-Jolley, R., Lewis, R., Fallman, R., & Mobasheri, A., (2010). The emerging chondrocyte channelome. *Frontiers in Physiology, 1*(10): 135. doi:10.3389/fphys.2010.00135

Beier, F., & Loeser, R. F., (2010). Biology and pathology of Rho GTPase, PI-3 kinase-Akt, and MAP kinase signaling pathways in chondrocytes. *Journal of Cell Biochemistry,* 110(3): 573-580.

Belluoccio, D., Etich, J., Rosenbaum, S., Frie, C., Grskovic, I., Stermann, J., & Brachvogel, B., (2010). Sorting of growth plate chondrocytes allows the isolation and characterization of cells of a defined differentiation status. *Journal of Bone and Mineral Research, 25*(6): 1267-1281. doi:10.1002/jbmr.30

Benítez-Rangel, E., López-Méndez, M., García, L., & Guerrero-Hernández, A., (2015). DIDS (4,4'-Diisothiocyanatostilbene-2,2'-disulfonate) directly inhibits caspase activity in HeLa cell lysates. *Cell Death Discovery, 1*(1): 15037. doi:10.1038/cddiscovery.2015.37

Bhattacharya, I., & Boje, K. M., (2004). GHB (gamma-hydroxybutyrate) carrier-mediated transport across the blood-brain barrier. *The Journal of Pharmacology and Experimental Therapeutics, 311*(1): 92-98.

Bianco, P., Cao, X., Frenette, P. S., Mao, J. J., Robey, P. G., Simmons, P. J., & Wang, C., (2013). The meaning, the sense and the significance: Translating the science of mesenchymal stem cells into medicine. *Nature Med*icine, 19(1): 35-42. doi:10.1038/nm.3028.

Bianco, P., Robey, P., & Simmons, P., (2008). Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell,* 2: 313-319.

Bigham-Sadegh, A., & Oryan, A., (2014). Basic concepts regarding fracture healing and the current options and future directions in managing bone fractures. *International Wound Journal, 12*(3): 238-47. doi:10.1111/iwj.12231

Bilodeau, K. M. D., (2006). Bioreactors for tissue engineering: focus on mechanical constraints. A comparative review. *Tissue Engineering,* 12: 2367-2383.

Binder, G., Huller, E., Blumenstock, G., & Schweizer, R., (2011). Auxology-based cut-off values for 381 biochemical testing of GH-secretion in childhood. *Growth Hormone and IGF Research,* 21(4): 212-218. DOI: 10.1016/j.ghir.2011.05.007

Binder, G., Wittekindt, N., & Ranke, M. B., (2007). Noonan syndrome: genetics and responsiveness to growth hormone therapy. *Hormone Research,* 67(Supplement 1): 45-49. doi:10.1159/000097552.

Bingham, P. J,. & Raisz, L. G., (1974). Bone growth in organ culture: effects of phosphate and other nutrients on bone and cartilage. *Calcified Tissue Research,* 14: 31-48.

Blair, H. C., & Athanasou, N. A., (2004). Recent advances in osteoclast biology and pathological bone resorption. *Histology and Histopathology, Cellular and Molecular Biology,* 19: 189-199.

Bleedorn, J. A., Sullivan, R., Lu, Y., Nemke, B., Kalscheur, V., & Markel, M. D., (2014). Percutaneous lovastatin accelerates bone healing but is associated with periosseous soft tissue inflammation in a canine tibial osteotomy model. *Journal of Orthopaedic Research, 32*(2): 210-216. doi:10.1002/jor.22502

Boassa, D., Stamer, W. D., & Yool, A. J., (2006). Ion channel function of aquaporin-1 natively expressed in choroid plexus. *Journal of Neuroscience, 26*(30): 7811-7819. doi:10.1523/JNEUROSCI.0525-06.2006

Bobick, B. E., & Kulyk, W. M., (2008). Regulation of cartilage formation and maturation by mitogen activated protein kinase signaling. *Birth Defects* Research Part C *Embryo Today,* 84(2): 131-154.

Bock, N., Riminucci, A., Dionigi, C., Russo, A., Tampieri, A., Landi, E., Goranov, V. A., Marcacci, M., & Dediu, V., (2010). A novel route in bone tissue engineering: magnetic biomimetic scaffolds. *Acta Biomaterialia,* 6: 786-796.

Bolgen, N., Yang, Y., Korkusuz, P., Guzel, E., El Haj, A. J., & Piskin, E., (2008). Three-Dimensional ingrowth of bone cells within biodegradable cryogel scaffolds in bioreactors at different regimes. *Tissue Engineering Part A,* 14: 1743-1750.

Bonnarens. F., & Einhorn, T., (1984): Production of a standard closed fracture in laboratory animal bone. *Journal of Orthopaedics Research,* 2: 97-101. DOI: 10.1002/jor.1100020115

Boskey, A. L., & Robey, P. G., (2013). The regulatory role of matrix proteins in mineralization of bone. In M. M. D. Robert, D. Feldman, D. W. Dempster, & M. Luckey, (Eds.), *Osteoporosis.* (pp. 235-258): Elsevier.

Boskey, A. L., (2013). Bone composition: relationship to bone fragility and antiosteoporotic drug effects. *BoneKEy Reports2* (International Bone & Mineral Society), 447: 1-11. doi:10.1038/bonekey.2013.181

Bosmans, F., Martin-Eauclaire, M. F., & Swartz, K. J., (2008). Deconstructing voltage sensor function and pharmacology in sodium channels. *Nature, 456*(7219): 202-8. doi:10.1038/nature07473

Boyce, B. F., & Xing, L., (2008). Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Archives of Biochemistry and Biophysics, 473*(2): 139-146. doi:10.1016/j.abb.2008.03.018

Boyle, W. J., Simonet, W. S., & Lacey, D. L., (2003). Osteoclast differentiation and activation. *Nature,* 423: 337-342. doi:10.1038/nature01658

Buenzli, P. R., & Sims, N. A., (2015). Quantifying the osteocyte network in the human skeleton. *Bone*, *75*: 144-50. doi:10.1016/j.bone.2015.02.016

Buenzli, P. R., (2015). Osteocytes as a record of bone formation dynamics: A mathematical model of osteocyte generation in bone matrix. *Journal of Theoretical Biology, 364*: 418-427. doi:10.1016/j.jtbi.2014.09.028

Bullis, B. L., Li, X., Rieder, C. V., Singh, D. N., Berthiaume, L. G., & Fliegel, L., (2002). Properties of the Na+/H+ exchanger protein. *European Journal of Biochemistry, 269*(19): 4887–4895. doi:10.1046/j.1432-1033.2002.03202.x

Burr, D. B., (2001). Bone material properties and mineral matrix contributions to fracture risk or age in women and men. *Journal of Musculoskeletal Neuronal Interaction,* 2: 201-204.

Burr, D. B., (2004). Anatomy and physiology of the mineralized tissues: Role in the pathogenesis of osteoarthrosis. *Osteoarthritis and Cartilage*, *12*, 20–30. doi:10.1016/j.joca.2003.09.016

Buschmann, J., Härter, L., Gao, S., Hemmi, S., Welti, M., Hild, N., Schneider, O. D., Stark, W. J., Lindenblatt, N., Werner, C. M. L., Wanner, G. A., & Calcagni, M., (2012). Tissue engineered bone grafts based on biomimetic nanocomposite PLGA/ amorphous calcium phosphate scaffold and human adipose-derived stem cells. *Injury*, 43: 1689-1697.

Bush, P. G., Pritchard, M., Loqman, M. Y., Damron, T., & Hall, A., C. (2010). A key role for membrane transporter NKCC1 in mediating chondrocyte volume increase in the mammalian growth plate. *Journal of Bone and Mineral Research , 25*(7): 1594-1603. doi:10.1002/jbmr.47

Bush, P. G., Parisinos, C. A., & Hall, A. C., (2008). The osmotic sensitivity of rat growth plate chondrocytes in situ; clarifying the mechanisms of hypertrophy. *Journal of Cell Physiology*, 214(3): 621-629.

Bustamante, J. J., Gonzalez, L., Carroll, C. A., Weintraub, S. T., Aguilar, R. M., Muñoz, J., Martinez, A. O., & Haro, L. S., (2009). O-Glycosylated 24-kDa human growth hormone (hGH) has a mucin-like biantennary disialylated tetrasaccharide attached at Thr-60. *Proteomics,* 9 (13): 3474–88. doi:10.1002/pmic.200800989.

Butterworth, M. B., (2010). Regulation of the epithelial sodium channel (ENaC) by membrane trafficking. *Biochimica et Biophysica Acta (BBA), Molecular Basis of Disease, 1802*(12): 1166-1177. doi:10.1016/j.bbadis.2010.03.010

Camerino, D. C., Desaphy, J. F., Tricarico, D., Pierno, S., & Liantonio, A., (2008). Therapeutic approaches to ion channel diseases. *Advances in Genetics,* 64: 81-145. doi:10.1016/S0065-2660(08)00804-3.

Camerino, D. C., Tricarico, D., & Desaphy, J. F., (2007). Ion Channel Pharmacology. *The Journal of the American Society for Experimental Neuro Therapeutics, 4*(4): 184-198. doi:10.1016/j.nurt.2007.01.013

Cameron, J. A Milner, D. J., Lee, J. S., Cheng, J., Fang, N. X., & Jasiuk, I. M., (2013). Employing the Biology of Successful Fracture Repair to Heal Critical Size Bone Defects. *Current Topics in Microbiology and Immunology,* 367: 113-132. DOI: 10.1007/82_2012_291

Carter-Su, C., Schwartz, J., & Argetsinger, L. S., (2015). Growth hormone signaling pathways. *Growth Hormone & IGF Research*, 2015 (10): 29-40. doi:10.1016/j.ghir.2015.09.002

Chagin, A. S., Karimian, E., Sundström, K., Eriksson, E., & Sävendahl, L., (2010). Catch-up growth after dexamethasone withdrawal occurs in cultured postnatal rat metatarsal bones. *The Journal of Endocrinology*, *204*(1): 21-9. doi:10.1677/JOE-09-0307

Chagin, A. S., Karimian, E., Zaman, F., Takigawa, M., Chrysis, D., & Sävendahl, L., (2007). Tamoxifen induces permanent growth arrest through selective induction of apoptosis in growth plate chondrocytes in cultured rat metatarsal bones. *Bone,* 40: 1415-1424.

Checa, S., Prendergast, P. J., & Duda, G. N., (2011). Inter-species investigation of the mechano-regulation of bone healing: Comparison of secondary bone healing in sheep and rat. *Journal of Biomechanics, 44*(7): 1237-1245. doi:10.1016/j.jbiomech.2011.02.074

Chellaiah, M. A., Soga, N., Swanson, S., Mcallister, S., Alvarez, U., Wang, D., & Hruska, K. A., (2000). Rho-A is critical for osteoclast podosome organization, motility, and bone resorption. *The Journal of Biological Chemistry,* 275(16): 11993-12002.

Chen, H., & Sun, D., (2005). The role of Na-K-Cl co-transporter in cerebral ischemia. *Neurological Research, 27*(3): 280-6. doi:10.1179/016164105X25243

Chen, Z., Wen, Z., & Bai, X., (2013). In vivo chick chrioallantoic membrane (CAM) angiogenesis assays. *Bio-Protocol,* 3(18): e193.

Cheung, K., Kaluarachi, K., Andrew, G., Lu, W., Chan, D., & Cheah, K., (2003). An externally fixed femoral fracture model for mice. *Journal of Orthopaedic Research, 21*(4): 685-690. doi:S0736026603000263

Chifflet, S., & Hernández, J., (2012). The plasma membrane potential and the organization of the actin cytoskeleton of epithelial cells. *International Journal of Cell Biology, 2012*: 121424. doi:10.1155/2012/121424

Childs, S. G., (2003). Stimulators of Bone Healing. *Orthopaedic Nursing*, *22*(6): 421-428.

Cho, T., Jonghoon, K., Soon-Keun, K., Keunhee, O., Jeong-ae, L., Dong-Sup, L., Jaejin, C., & Seung, B. P., (2012). A potent small-molecule inducer of chondrogenic differentiation of human bone marrow-derived mesenchymal stem cells. *Chemical Science,* 3 (10): 3071. doi:10.1039/c2sc20362f.

Chung, U. I., (2004). Essential role of hypertrophic chondrocytes in endochondral bone development. *Endocrine Journal*, *51*(1): 19–24. doi:10.1507/endocrj.51.19

Claes, L., Maurer-Klein, N., Henke, T., Gerngross, H., Melnyk, M., & Augat, P., (2006). Moderate soft tissue trauma delays new bone formation only in the early phase of fracture healing. *Journal of Orthopaedic Research,* 24: 1178-1185. DOI 10.1002/jor.20173

Clark, R. B., Kondo, C., Belke, D. D., & Giles, W. R., (2011). Two-pore domain K^+ channels regulate membrane potential of isolated human articular chondrocytes. *The Journal of Physiology, 589*(Pt 21): 5071-89. doi:10.1113/jphysiol.2011.210757

Clarke, B. (2008). Normal bone anatomy and physiology. *Clinical Journal of the American Society of Nephrology, 3 (Suppl 3)*: S131-9. doi:10.2215/CJN.04151206

Clendenning, D. E., & Mortlock, D. P., (2012). The BMP ligand Gdf6 prevents differentiation of coronal suture mesenchyme in early cranial development. *PloS One*, *7*(5): e36789. doi:10.1371/journal.pone.0036789

Colnot, C., & Alliston, T., (2010). Tissue Interaction in Long Bones. In: F., Bronner, M. C., Farach-Carson, & H. I., Roach (Eds.), *Bone and Development, Topics in Bone Biology* (Vol. 6. pp. pp. 25-38): Springer

Colnot, C., Lu, C., Hu, D., & Helms, J. A., (2004). Distinguishing the contributions of the perichondrium, cartilage, and vascular endothelium to skeletal development. *Developmental Biology*, *269*(1): 55-69. doi:10.1016/j.ydbio.2004.01.011

Colombo, J. S., Howard-Jones, R. A., Young, F. I., Waddington, R. J., Errington, R. J., & Sloan, A. J., (2015). A 3D *ex vivo* mandible slice system for longitudinal culturing of transplanted dental pulp progenitor cells. *Cytometry Part A,* 87(7): 1-8.

Concepcion, A. R., Lopez, M., Ardura-Fabregat, A., & Medina, J. F., (2014). Role of AE2 for pHi regulation in biliary epithelial cells. *Frontiers in Physiology, 4*: 1–7. doi:10.3389/fphys.2013.00413

Contreras, F. X., Sánchez-Magraner, L., Alonso, A., & Goñi, F. M., (2010). Transbilayer (flip-flop) lipid motion and lipid scrambling in membranes. *FEBS Letters, 584*(9): 1779-86. doi:10.1016/j.febslet.2009.12.049

Cooper, K. L., Oh, S., Sung, Y., Dasari, R. R., Marc, W., & Tabin, C. J., (2013a). Multiple phases of chondrocyte enlargement underlie differences in skeletal proportions. *Nature, 495*(7441): 375-378. doi:10.1038/nature11940.

Cooper, K. L., Oh, S., Sung, Y., Dasari, R. R., Marc, W., & Tabin, C. J., (2013b). Skeletons growth through three phases of chondrocyte enlargement. *Bonekey Report*, 2:379.

Coppini, R., Ferrantini, C., Mazzoni, L., Sartiani, L., Olivotto, I., Poggesi, C., & Mugelli, A., (2013). Regulation of intracellular Na 1 in health and disease : pathophysiological mechanisms and implications for treatment. *Global Cardiology Science and Practice,* 2013(3): 222–242. doi: 10.5339/gcsp.2013.30

Cottrell, J., & O'Connor, J. P., (2010). Effect of non-steroidal anti-inflammatory drugs on bone healing. *Pharmaceuticals, 3*(5): 1668-1693. doi:10.3390/ph3051668

Counillon, L., & Pouysse, J., (2000). The expanding family of eucaryotic Na/H exchangers. *Biochemistry, 275*(33): 1-4. doi:10.1074/jbc.275.1.1

Craft, A. M., Ahmed, N., Rockel, J. S., Baht, G. S., Alman, B., Kandel, R., & Keller, G. M., (2013). Specification of chondrocytes and cartilage tissues from embryonic stem cells. *Development, 140*(12): 2597-610. doi:10.1242/dev.087890

Crichton, R. R., Lallemand, F., Psalti, I. M. S., & Ward, R. J., (2008). Biological inorganic chemistry an introduction. *Elsevier*. 66-67.

Crockett, J. C., Rogers, M. J., Coxon, F. P., Hocking, L. J., & Helfrich, M. H., (2011). Bone remodelling at a glance. *Journal of Cell Science, 124*(7): 991-998. doi:10.1242/jcs.063032

Crombrugghe, B. D., Lefebvre, V., & Nakashima, K., (2001). Regulatory mechanisms in the pathways of cartilage and bone formation *Current Opinion in Cell Biology,* 13:721-727.

Currey, J. D., (2002). Bones: structure and mechanics. Princeton University Press, Princeton, N.J. pp. 1-26.

Curtin, P., Youm, H., & Salih, E., (2012).Three-dimensional cancer-bone metastasis model using *ex vivo* co-cultures of live calvarial bones and cancer cells. *Biomaterials,* 33(4): 1065-1078. doi:10.1016/j.biomaterials.2011.10.046.

Dai, J., & Rabie, A. B. M., (2007). VEGF: an essential mediator of both angiogenesis and endochondral Ossification. *Journal of Dental Research,* 86: 937-950.

Dallas, S. L., & Bonewald, L. F., (2011). Dynamics of the transition from osteoblast to osteocyte. *Developmental Dynamics*, (816): 437-443. doi:10.1111/j.1749-6632.2009.05246.x.

David, V., Guignandon, A., Martin, A., Malaval, L., Lafage-Proust, M. H., Rattner, A., Mann, V., Noble, B., Jones, D., & Vico, L., (2008). *Ex vivo* bone formation in bovine trabecular bone cultured in a dynamic 3D bioreactor is enhanced by compressive mechanical strain. *Tissue Engineering Part A,* 14 (1): 117-126.

Davies, C. M., Jones, D. B., Stoddart, M. J., Koller, M., Smith, E., Archer, A. W., & Richards, R. G., (2006). Mechanically loaded *ex vivo* bone culture system 'Zetos': systems and culture preparation. *European Cells and Material,* 11: 57-75.

Davison, K. S., Siminoski, K., Adachi, J. D., Hanley, D. A., Goltzman, D., Hodsman, A. B., Josse, R., Kaiser, S., Olszynski, W. P., Brown, J. P., (2006). Bone strength: the whole is greater than the sum of its parts. *Seminar in Arthritis Rheumatism,* 36:22-31.2006. doi.org/10.1016/j.semarthrit.2006.04.002

De la Torre, N. G., Buley, I., Wass, J. A. H., Turner, H. E., (2006). Angiogenesis and lymphangiogenesis in thyroid proliferative lesions: relationship to type and tumour behaviour. *Endocrine-Related Cancer* 13: 931-944. DOI:10.1677/erc.1.01210

De-Giacomo, A., Morgan, E. F., & Gerstenfeld, L. C., (2014). Generation of closed transverse fractures in small animals. *Methods in Molecular Biology,* 1130: 35-44. doi:10.1007/978-1-62703-989-5_3.

DeLise, a. M., Fischer, L., & Tuan, R. S., (2000). Cellular interactions and signaling in cartilage development. *Osteoarthritis and Cartilage, 8*(5): 309-334. doi:10.1053/joca.1999.0306

Dempster, D. W., (2006). Anatomy and functions of the adult skeleton. In M. J. Favus, (Eds.), *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, (pp. 7-11): American Society for Bone and Mineral Research

Dempster,D., Compston, J. E., Drezner, M. K., Glorieux, F. H., Kanis, J. A, Malluche, H., Meunier, P. J. Ott, S. M., Recker, R. R. & Parfitt, A. M. (2013). Standardized Nomenclature, Symbols, and Units for Bone Histomorphometry: A 2012 Update of the Report of the ASBMR Histomorphometry Nomenclature Committee. *Journal of Bone and Mineral Research,* 28(1):1-16.

Deryugina, E. I., & Quigley, J. P., (2008). Chick embryo chorioallantoic membrane models to quantify angiogenesis induced by inflammatory and tumor cells or purified effector molecules. *Methods in Enzymology,* 444: 21-41.

Desjardin, C., Charles, C., Benoist-Lasselin, C., Riviere, J., Gilles, M., Chassande, O., & Schibler, L., (2014). Chondrocytes play a major role in the stimulation of bone growth by thyroid hormone. *Endocrinology, 155*(8): 3123-3135. doi:10.1210/en.2014-1109

Desmarchelier, M., (2012). Evaluation of a fracture pain model in domestic pigeons (Columba livia). *American Journal of Veterinary Research*, *73*(3): 353-360. doi:10.2460/ajvr.73.3.353

Dettmeyer, R. B., (2011). Staining techniques and Microscopy, In R. B. Dettmeyer (Ed.), *Forensic Histopathology Fundamental and Perpectives*, (pp. 17-35): *Springe*

Devaux, P. F., & Morris, R., (2004). Transmembrane asymmetry and lateral domains in biological membranes. *Traffic,* 5: 241-246. doi:10.1111/j.1600-0854.2004.00170.x

Dimitriou, R., Jones, E., McGonagle, D., & Giannoudis, P. V., (2011). Bone regeneration: current concepts and future directions. *BMC Medicine,* 9: 66. DOI**:** 10.1186/1741-7015-9-66.

Dobie, R., Ahmed, S. F., Staines, K. A., Pass, C. Jasim, S., Macrae, V. E. & Farquharson, C. (2015). Increased linear bone growth by GH in the absence of SOCS2 is independent of IGF-1. *Journal of Cell Physiology,* 230: 2796-2806.

Dobson, J., Cartmell, S. H., Keramane, A., & El Haj, A. J., (2006). Principles and Design of a novel magnetic force mechanical conditioning bioreactor for tissue engineering, stem cell conditioning, and dynamic *in vitro* screening. *IEEE Trans Nanobioscience,* 5: 173-177.

Dohle, D. S., Pasa, S. D., Gustmann, S., Laub, M., Wissler, J. H., Jennissen, H. P., & Dünker, N., (2009). Chick *ex ovo* culture and *ex ovo* CAM assay: how it really works. *Journal of Visualized Experiment,* 33: e1620.

Downey, P. A., & Siegel, M. I., (2006). Bone biology and the clinical. *Journal of Physical Therapy, 86*(1): 77-91.

Dowthwaite, G. P., Bishop, J. C., Redman, S. N., Khan, I. M., Rooney, P., Evans, D. J. R., & Archer, C. W., (2004). The surface of articular cartilage contains a progenitor cell population. *Journal of Cell Science, 117*(Pt 6): 889-897. doi:10.1242/jcs.00912

Drissi, H., & Paglia, D. N., (2015): Surgical procedures and experimental outcomes of closed fractures in rodent models. In J. J., Westendorf, & J. M., Walker, (Eds.), *Osteoporosis and Osteoarthritis Methods in Molecular Biology* (Vol. 1226, pp. 193-211): Springer. doi:10.1007/978-1-4939-1619-1

Drosos, G. I., Bishay, M., Karnezis, I. A., & Alegakis, A. K., (2006). Factors affecting fracture healing after intramedullary nailing of the tibial diaphysis for closed and grade I open fractures. *The Journal of Bone and Joint Surgery. British Volume, 88*(2): 227-231. doi:10.1302/0301-620X.88B2.16456

Eagle, H. (1955). Nutritional needs of mammalian cells in tissue culture. *Science*, 122: 501-504.

Eastaugh-Waring, S. J., Joslin, C. C., Hardy, J. R. W., & Cunningham, J. L., (2009). Quantification of fracture healing from radiographs using the maximum callus index. *Clinical Orthopaedic Related Research,* 467: 1986-1991. DOI 10.1007/s11999-009-0775-0

Egger, E., & Pluhar, E., (2015). Enhancement of fracture healing. In N. J., Bojrab, & E., Monnet, (Eds.), *Mechanism of disease in Small Animal Surgery*, (Vol. 3, pp. 1-10): Teton NewMedia

Eide, S., (2002). Studies of human osteoblast-like cells - effects of growth hormone and steroids. *Electronic Journal of International Federation of Clinical Chemistry and Laboratory Medicine,* 13(4): 20-25.

Einhorn, T. A., & Gerstenfeld, L. C., (2015). Fracture healing: mechanisms and interventions. *Nature Reviews Rheumatology,* 11: 45-54. doi:10.1038/nrrheum.2014.164

El Haj, A. J., & Cartmell, H., (2010). Bioreactors for bone tissue engineering. *Proceeding of Institution of Mechanical Engineering H,* 224: 1523-1532.

Emara, K. M., (2015). Recent biological trends in management of fracture non-union. *World Journal of Orthopedics, 6*(8): 623. doi:10.5312/wjo.v6.i8.623

Emons, J., Chagin, A. S., Hultenby, K., Zhivotovsky, B., Wit, J. M., Karperien, M., & vendahl, L., (2009). Epiphyseal fusion in the human growth plate does not involve classical apoptosis. *Pediatric Research, 66*(6): 654-659. doi:10.1203/PDR.0b013e3181beaa8c

Enishi, T., Yukata, K., Takahashi, M., Sato, R., Sairyo, K., & Yasui, N., (2014). Hypertrophic chondrocytes in the rabbit growth plate can proliferate and differentiate into osteogenic cells when capillary invasion is interposed by a membrane filter. *PLoS ONE,* 9(8): e104638.

Enomoto, H., Shiojiri, S., Hoshi, K., Furuichi, T., Fukuyama, R., Yoshida, C. A., & Komori, T., (2003). Induction of osteoclast differentiation by Runx2 through receptor activator of nuclear factor-kB ligand (RANKL) and osteoprotegerin regulation and partial rescue of osteoclastogenesis in Runx2$^{-/-}$ mice by RANKL transgene. *The Journal of Biological Chemistry,* 278(26): 23971-23977. doi:10.1074/jbc.M302457200

Eraly, S., Bush, K. T., Sampogna, R. V, Bhatnagar, V., & Nigam, S. K., (2004). The molecular pharmacology of organic anion transporters: from DNA to FDA? *Molecular Pharmacology, 65*(3): 479-87. doi:10.1124/mol.65.3.479

Erben, R. G., (2015). Hypothesis: coupling between resorption and formation in cancellous bone remodeling is a mechanically controlled event. *Frontiers in Endocrinology, 6*(82). doi:10.3389/fendo.2015.00082

Eriksen, E. F., (2010). Cellular mechanisms of bone remodeling. *Reviews in Endocrine and Metabolic Disorders, 11*(12): 219-227. doi:10.1007/s11154-010-9153-1

Estai, M. A., Soelaiman, N. I., Shuid, A. N., Das, S., Ali, A. M., & Suhaimi, F. H., (2011). Histological changes in the fracture callus following the administration of water extract of Piper sarmentosum (Daun Kadok) in estrogen-deficient rats. *Iranian Journal of Medical Sciences, 36*(4): 281-288.

Farnum, C. E., Lee, R., O'Hara, K., & Urban, J. P. G., (2002). Volume increase in growth plate chondrocytes during hypertrophy: the contribution of organic osmolytes. *Bone, 30*(4): 574-581. doi:10.1016/S8756-3282(01)00710-4

Fattore, A. Del, Teti, A., & Rucci, N., (2008). Osteoclast receptors and signaling. *Archives of Biochemistry and Biophysics,* 473: 147-160. doi:10.1016/j.abb.2008.01.011

Fedchenko, N., & Reifenrath, J., (2014). Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue -A review. *Diagnostic Pathology,* 9: 221. doi:10.1186/s13000-014-0221-9

Feflea, S., Cimpean, A. M., Ceausu, R.A., Gaje, P., & Raica, M., (2012). Effects of antibodies to EG-VEGF on angiogenesis in the chick embryo chorioallantoic membrane. *In Vivo,* 26: 793-797.

Fell, H. B., & Robison, R., (1929). The growth, development and phosphatase activity of embryonic avian femora and limb-buds cultivated *In Vitro*. *Biochemistry Journal,* 23: 767-784.

Fell, H. B., (1956). Biochemistry and physiology of bone. In G. H., Bource (Ed.) New York Academic Press. p. 402.

Fernandes, A. M., Herlofsen, S. R., Karlsen, T. A., Küchler, A. M., Fløisand, Y., & Brinchmann, J. E., (2013). Similar properties of chondrocytes from osteoarthritis joints and mesenchymal stem cells from healthy donors for tissue engineering of articular cartilage. *PLoS ONE, 8*(5): e62994. doi:10.1371/journal.pone.0062994

Fitzpatrick, N., Bertran, J. & Solano, M. A. (2015): Sliding Humeral Osteotomy: Medium-Term Objective Outcome Measures and Reduction of Complications with a Modified Technique. *Veterinary Surgery* 44: 137-149. DOI:10.1111/j.1532-950X.2014.12213.x

Flatman, P. W., (2002). Regulation of Na–K–2Cl cotransport by phosphorylation and protein–protein interactions. *Biochimica et Biophysica Acta–Biomembranes, 1566*(1-2): 140-151. doi:10.1016/S0005-2736(02)00586-2

Fliegel, L., (2005). The Na+/H+ exchanger isoform 1. *International Journal of Biochemistry and Cell Biology, 37*(1): 33–37. doi:10.1016/j.biocel.2004.02.006

Fliegel, L., (2009). Regulation of the Na + / H + exchanger in the healthy and diseased myocardium, *Expert Opinion on Therapeutic Targets,* 13(1): 55-68. doi: 10.1517/14728220802600707

Florencio-Silva, R., Rodrigues, G., Sasso, S., Sasso-Cerri, E., Simões, M. J., & Cerri, P. S., (2015). Biology of bone tissue: structure, function, and factors that influence bone cells. *BioMed Research Internationa,l 2015*: 1-17. doi:10.1155/2015/421746

Fraser, J. A., Middlebrook, C. E., Usher-Smith, J. A., Schwiening, C. J., & Huang, C. L., (2005). The effect of intracellular acidification on the relationship between cell volume and membrane potential in amphibian skeletal muscle. *The Journal of Physiology, 563*(Pt 3): 745-764.

Friedrich, B., Matskevich, I., & Lang, F., (2006). Cell volume regulatory mechanisms: An Introduction. In F., Lang, (Ed.), *Mechanisms and Significance of Cell Volume Regulation Contributions to Nephrology.* (Vol. 152, pp. 1-8): Basel, Karger. doi:10.1159/000096284

Frink, M., Andruszkow, H., Zeckey, C., Krettek, C., & Hildebrand, F., (2011). Experimental trauma models: An update. *Journal of Biomedicine and Biotechnology, 2011*. doi:10.1155/2011/797383

Frische, S., Zolotarev, A. S., Kim, Y. H., Praetorius, J., Alper, S., Nielsen, S., & Wall, S. M. (2004). AE2 isoforms in rat kidney: immunohistochemical localization and regulation in response to chronic NH4Cl loading. *American Journal of Physiology. Renal Physiology, 286*(6): F1163- F1170. doi:10.1152/ajprenal.00409.2003

Fritsch, A., & Hellmich, C., (2007). Universal microstructural patterns in cortical and trabecular, extracellular and extravascular bone materials: Micromechanics-based prediction of anisotropic elasticity. *Journal of Theoretical Biology,* 244: 597-620.

Gaillard, P. J., (1953). Growth and differentiation of explanted tissues. *International Review of Cytology,* 2: 331-401.

Garcia, P., Holstein, J. H., Histing, T., Burkhardt, M., Culemann, U., Pizanis, A., & Menger, M. D., (2008). A new technique for internal fixation of femoral fractures in mice: Impact of stability on fracture healing. *Journal of Biomechanics, 41*(8): 1689-1696. doi:10.1016/j.jbiomech.2008.03.010

Gaston, M., & Simpson, A., (2007). Inhibition of fracture healing. *The Journal of Bone and Joint Surgery. British Volume, 89*(12): 1553-1560. doi:89-B/12/1553 [pii]10.1302/0301-620X.89B12.19671

Gdyczynski, C. M., Manbachi, A., Hashemi, S. M., Lashkari, B., & Cobbold, R. S. C., (2014). On estimating the directionality distribution in pedicle trabecular bone from micro-CT Images. *Physiological Measurement,* 35: 2415-2428.

Georgess, D., Machuca-gayet, I., Blangy, A., & Jurdic, P., (2014). Podosome organization drives osteoclast- mediated bone resorption. *Cell Adhesion and Migration, 8*(3): 192-204.

Geris, L., Gerisch, A., Sloten, J. Vander, Weiner, R., & Oosterwyck, H. Van., (2008). Angiogenesis in bone fracture healing: A bioregulatory model. *Journal of Theoretical Biology, 251*(1): 137-158. doi:10.1016/j.jtbi.2007.11.008

Gerstenfeld, L. C., Cullinane DM, Barnes GL, Graves, D. T., & Einhorn, T. A., (2003). Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. *Journal of Cell Biochemistry,* 88: 873-84. DOI 10.1002/jcb.10435

Giacomini, K. M., & Sugiyama, Y., (2006). Membrane Transport and Drug response. In L. L., Brunto, J. S., Lazo, & K. L., Parker, (Eds.), *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Vol. 11. pp. 41-70): McGraw-Hill Medical Publishing Division.

Giannoudis, P., Psarakis, S., & Kontakis, G., (2007). Can we accelerate fracture healing? A critical analysis of the literature. *Injury, 38 Suppl 1*(1): S81-S89. doi:10.1016/j.injury.2007.02.013

Gierer, P., Mittlmeier, T., Bordel, R., Schaser, K.-D., Gradl, G., & Vollmar, B., (2005). Selective cyclooxygenase-2 inhibition reverses microcirculatory and inflammatory sequelae of closed soft-tissue trauma in an animal model. *The Journal of Bone and Joint Surgery. American Volume, 87*(1): 153-60. doi:10.2106/JBJS.C.01510

Gnecchi, M., & Melo, L. S., (2009). Bone marrow-derived mesenchymal stem cells isolation, expansion, characterization, viral transduction, and production of conditioned media. In J., Audet, & W. L., Stanford, (Eds.), *Stem Cell in Regenerative Medicine: Methods and Protocols*. (Vol. 482. pp. 281-294): Springer.

Goldhaber, P., (1966). Remodeling of bone in tissue culture. *Journal of Dental Research,* 45 (suppl. 3): 490-499.

Gomez, S., (2002). The Discoverer of trabecular bone. *Endocrine,* 17(1): 3-4.

Gómez-Barrena, E., Rosset, P., Lozano, D., Stanovici, J., Ermthaller, C., & Gerbhard, F., (2015). Bone fracture healing: Cell therapy in delayed unions and nonunions. *Bone, 70*: 93-101. doi:10.1016/j.bone.2014.07.033

Gong, H., Zhu, D., Gao, J., Linwei, L. V., & Zhang, Z., (2010). An adaptation model for trabecular bone at different mechanical levels. *Biomedical Engineering On-Line,* 9:32-48.

Gonzalez-Begne, M., Nakamoto, T., Nguyen, H., Stewart, A. K., Alper, S. L., & Melvin, J. E., (2007). Enhanced formation of a HCO3 transport metabolon in exocrine cells of Nhe1/ mice. *The Journal of Biological Chemistry,* 282(48): 35125-35132. DOI 10.1074/jbc.M707266200

Goodship, A., (2004). Mechanical modulation of fracture healing and Implication of skeletal tissue engineering. In H., Petiti, & R., Quartro, (Eds.), *Engineered Bone*. Eurekah.com

Green, D., Walsh, D., Yang, X., Mann, S., & Oreffo, R. O. C., (2004). Stimulation of human bone marrow stromal cells using growth factor encapsulated calcium carbonate porous microspheres. *Journal of Material Chemistry,* 14: 2206-2212.

Greger, R., (1996a). The cell and it membrane. In R., Greger, & U., Windhorst, (Eds.), *Comprehensive Human Physiology: From Cellular Mechanisms to Integration*. (Vol. 1. pp. 79-93) Springer.

Greger, R., (1996b). Cellular membrane transport mechanisms. In R., Greger, & U., Windhorst, (Eds.), *Comprehensive Human Physiology: From Cellular Mechanisms to Integration*. (Vol. 1. pp. 149-171): Springer.

Griffon, D. J., (2005). Fracture healing In A., Johnson, (Ed.), *AO Principle of Fracture Management in Dog and Cat*. (Vol. 1. pp. 73-92): Thieme Medical Publishers.

Griffon, D. J., (2012). Secondary bone healing. In N. J., Bojrab, & E., Monnet, (Eds.), *Mechanism of disease in Small Animal Surgery*. (Vol. 3. pp. 1-10): Teton NewMedia

Griffon, D. J., (2015). Primary bone healing. In N. J., Bojrab, & E., Monnet, (Eds.), *Mechanism of disease in Small Animal Surgery*. (Vol. 3. pp. 1-7)

Gruber, R., Koch, H., Doll, B. a., Tegtmeier, F., Einhorn, T., & Hollinger, J. O., (2006). Fracture healing in the elderly patient. *Experimental Gerontology, 41*(11): 1080-1093. doi:10.1016/j.exger.2006.09.008

Gu, J., Lu, Y., Li, F., Qiao, L., Wang, Q., Li, N., Borgia, J. N., Deng, Y., Lei, G., & Zheng, Q., (2014). Identification and characterization of the novel Col10a1 regulatory mechanism during chondrocytes hypertrophic differentiation. *Cell Death and Disease,* (2014) 5: e1469; doi:10.1038/cddis.2014.444

Guenou, H., (2005). A role for fibroblast growth factor receptor-2 in the altered osteoblast phenotype induced by twist haploinsufficiency in the Saethre-Chotzen syndrome. *Human Molecular Genetics, 14*(11): 1429-1439. doi:10.1093/hmg/ddi152

Hadjidakis, D. J., & Androulakis, I. I., (2006). Bone remodeling. *Annals of the New York Academy of Sciences, 1092*(1): 385–396. doi:10.1196/annals.1365.035

Haffner-Luntzer, M., Heilmann, A., Rapp, A. E., Beie, S., Schinke, T., Amling, M., Ignatius, A., Liedert, A. (2014). Midkine-Deficiency Delays Chondrogenesis during the Early Phase of Fracture Healing in Mice. *PLoS ONE,* 9(12): e116282.

Hak, D. J, Stewart, R. L., & Hazelwood, S. J., (2006): Effect of low molecular weight heparin on fracture healing in a stabilized rat femur fracture model. *Journal of Orthopaedics Research,* 24: 645-652. DOI 10.1002/jor.20090

Hall, B., (1981). Intracellular and extracellular control of the differentiation of cartilage and bone. *Histochemistry Journal,* 13: 599-614.

Hall, K. C., Hill, D., Otero, M., Plumb, D., Froemel, D., Dragomir, C. L., & Blobel, C. P., (2013). ADAM17 controls endochondral ossification by regulating terminal differentiation of chondrocytes. *Molecular and Cellular Biology, 33*(16): 3077-90. doi:10.1128/MCB.00291-13

Hamann, S., Herrera-Perez, J. J., Bundgaard, M., Alvarez-Leefmans, F. J., & Zeuthen, T., (2005). Water permeability of Na^+-K^+-$2Cl^-$ cotransporters in mammalian epithelial cells. *The Journal of Physiology, 568*(Pt 1): 123-135.

Hannemann, A., & Flatman, P. W., (2011). Phosphorylation and transport in the Na-K-2Cl cotransporters, NKCC1 and NKCC2A, compared in HEK-293 cells. *PLoS ONE, 6*(3): e17992. doi:10.1371/journal.pone.0017992

Harada, M., Miyahara, T., Miyata, M. I., Tomita, G. M., Ikemoto , S., Higuchi, S., Otomo, H., Kozuka, N., & Ikekawa, N., (1992). Effects on cultured neonatal mouse calvaria of 1α,25-dihydroxyvitaminD_3,26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_3 and 26,26,26,27,27,27-hexafluoro-1α,23S,25-trihydroxyvitamin D_3. *Bone,* 18(1): 41-49.

Hardin, D. S., Adams-Huet, B., Brown, D., Chatfield, B., Dyson, M., Ferkol, T., Howenstine, M., Prestidge, C., Royce, F., Rice, J., Seilheimer, D. K., Steelman, J., & Shepherds, R., (2006). Growth hormone treatment improves growth and clinical status in prepubertal children with cystic fibrosis: results of a multicenter randomized controlled trial. *Journal of Clinical Endocrinology and Metabolism,* 91: 4925-4929.

Hardin, D. S., Kemp, S. F., & Allen, D. B., (2007). Twenty years of recombinant human growth hormone in children: relevance to pediatric care providers. *Clinical Pediatric,* 46: 279-86.

Harguindey, S., Orive, G., Luis Pedraz, J., Paradiso, A., & Reshkin, S. J., (2005). The role of pH dynamics and the Na+/H+ antiporter in the etiopathogenesis and treatment of cancer. two faces of the same coin--one single nature. *Biochimica et Biophysica Acta, 1756*(1): 1-24. doi:10.1016/j.bbcan.2005.06.004

Havaldar, R., Pilli, S. C., & Putti, B. B., (2012). Effects of ageing on bone mineral composition and bone strength. *Journal of Dental and Medical Sciences, 1*(3): 12-16.

Hayes, K. E., Raucci, J., Gades, N. M., & Toth, L., (2000). An evaluation of analgesic regimens for abdominal surgery in mice. *Contemporary Topics in Laboratory Animal Science, 39*(6): 18-23.

Heaney, R. P., & Layman, D. K., (2008). Amount and type of protein influences bone health. *American Journal of Clinical Nutrition, 87*(suppl): 1567s-1570s.

Henstock, J. R., Rotherham, M., Rose, J. B., & El Haj, A. J., (2013). Cyclic hydrostatic pressure stimulates enhanced bone development in the foetal chick femur *in vitro*. *Bone,* 53: 468-477.

Hirose, S., Li, M., Kojima, T., de Freitas, P. H. L., Ubaidus, S., Oda, K., & Amizuka, N., (2007). A histological assessment on the distribution of the osteocytic lacunar canalicular system using silver staining. *Journal of Bone and Mineral Metabolism, 25*(6): 374-82. doi:10.1007/s00774-007-0764-x

Histing, T., Garcia, P., Matthys, R., Leidinger, M., Holstein, J. H., Kristen, A., & Menger, M. D., (2010). An internal locking plate to study intramembranous bone healing in a mouse femur fracture model. *Journal of Orthopaedic Research, 28*(3): 397–402. doi:10.1002/jor.21008

Hoffmann, E. K., Lambert, I. H., & Pedersen, S. F., (2009). Physiology of cell volume regulation in vertebrates. *Physiology Review,* 89: 193-277. doi:10.1152/physrev.00037.2007

Holappaa, K., Mun‹ozb, M. T., Egeab, G., & Kellokumpu, S., (2004). The AE2 anion exchanger is necessary for the structural integrity of the golgi apparatus in mammalian cells. *FEBS Letters,* 564: 97-103.

Hollinger, J. O., (2005). Bone dynamics: morphogenesis, growth modeling, and Remodeling: In J. R., Lieberman, & G. E., Friedlaender, (Eds.), *Bone Regeneration and Repair Biology and Clinical Applications.* (Vol. 1, pp. 1-19): Sringer. doi. 10.1385/1-59259-863-3:001

Holstein, J. H., Matthys, R., Histing, T., Becker, S. C., Fiedler, M., Garcia, P., & Menger, M. D., (2009). Development of a stable closed femoral fracture model in mice. *The Journal of Surgical Research, 153*(1): 71-5. doi:10.1016/j.jss.2008.02.042

Hosogi, S., Miyazaki, H., Nakajima, K. I., Ashihara, E., Niisato, N., Kusuzaki, K., & Marunaka, Y., (2012). An inhibitor of Na+/H+ exchanger (NHE), ethyl-isopropyl amiloride (EIPA), diminishes proliferation of MKN28 human gastric cancer cells by decreasing the cytosolic Cl- concentration via DIDS-sensitive pathways. *Cellular Physiology and Biochemistry, 30*(5): 1241–1253. doi:10.1159/000343315

Houghton, G. R., & Deke, S., (1979). The periosteal control of long bone growth: an experimental study in the rat. *Acta Orthopaedica Scandinavia,* 50: 635-637.

Houston, D. A., Staines, K. A., MacRae, V. E. & Farquharson, C. (2016). Culture of murine embryonic metatarsals: A physiological model of endochondral ossification. *Journal of Visualized Experiment,* (118): e54978.

Hu, J., Qu, J., Xu, D., Zhang, T., Qin, L., & Lu, H., (2014). Combined application of low-intensity pulsed ultrasound and functional electrical stimulation accelerates bone-tendon junction healing in a rabbit model. *Journal of Orthopaedic Research, 32*(2): 204-209. doi:10.1002/jor.22505

Huang, J., Shan, J., Kim, D., Liao, J., Evagelidis, A., Alper, S. L., & Hanrahan, J. W., (2012). Basolateral chloride loading by the anion exchanger type 2: role in fluid secretion by the human airway epithelial cell line Calu-3. *The Journal of Physiology, 590*(21): 5299–5316. doi:10.1113/jphysiol.2012.236919

Hughes, S., Dobson, J., & El Haj, A. J., (2007). Magnetic targeting of mechanosensors in bone cells for tissue engineering applications. *Journal of Biomechanical Engineering,* 40 Suppl 1: S96-S104.

Hwang, J. M., Kao, S. H., Hsieh, Y. H., Li, K. L., Wang, P. H., Hsu, L. S., & Liu, J. Y., (2009). Reduction of anion exchanger 2 expression induces apoptosis of human hepatocellular carcinoma cells. *Molecular and Cell Biochemistry, 327*(1-2): 135-144. doi:10.1007/s11010-009-0051-3

Isachenko, V., Mallmann, P., Petrunkina, A. M., Rahimi, G., Nawroth, F., Hancke, K., Felberbaum, R., Genze, R., Damjanoski, L., & Isachenko, E., (2012). Comparison of in vitro- and chorioallantoic membrane (CAM)-culture systems for cryopreserved medulla-contained human ovarian tissue. *PLoS ONE,* 7(3): e32549.

Isaksson, H., Comas, O., van Donkelaar, C. C., Mediavilla, J., Wilson, W., Huiskes, R., & Ito, K., (2007). Bone regeneration during distraction osteogenesis: Mechano-regulation by shear strain and fluid velocity. *Journal of Biomechanics, 40*(9): 2002-2011. doi:10.1016/j.jbiomech.2006.09.028

Ishida, N., Hayashi, K., Hoshijima, M., Ogawa, T., Koga, S., Miyatake, Y., & Takeya, T., (2002). Large scale gene expression analysis of osteoclastogenesis in vitro and elucidation of NFAT2 as a key regulator. *Journal of Biological Chemistry, 277*(43): 41147-41156. doi:10.1074/jbc.M205063200

Jackson, R. W., Reed, C. A., Israel, J. A., Abou-Keer, F. K., & Garside, H., (1970): Production of a standard experimental fracture. *Canadian Journal of Surgery,* 13: 415-420.

Jagodzinski, M., & Krettek, C., (2007): Effect of mechanical stability on fracture healing -an update. *Injury-International Journal of the Care of the Injured,* 38S1: S3-S10. doi:10.1016/j.injury.2007.02.005

Jahagirdar, R., & Scammell, B. E., (2009). Principles of fracture healing and disorders of bone union. *Surgery, 27*(2): 63-69. doi:10.1016/j.mpsur.2008.12.011

Jahani, M., Genever, P. G., Patton, R. J., Ahwal, F., & Fagan, M. J., (2012). The effect of osteocyte apoptosis on signalling in the osteocyte and bone lining cell network: A computer simulation. *Journal of Biomechanics, 45*(16): 2876-2883. doi:10.1016/j.jbiomech.2012.08.005

Jaitovich, A., & Bertorello, A. M., (2006). Na+, K+ -ATPase: an indispensable ion pumping-signaling mechanism across mammalian cell membranes. *Seminars in Nephrology, 26*(5): 386-92.

James, A. W., (2013). Review of signaling pathways governing MSC osteogenic and adipogenic differentiation. *Scientifica,* 2013: 684736. doi:10.1155/2013/684736.

Janezic, G., Windi, E. E., Haxhija, E. Q., Stradner, M., Frohlich, E., & Weinberg, A. M., (2010). Proliferation analysis of the growth plate after diaphyseal midshaft fracture by 5'-bromo-o2'-deoxy-uridine. *Virchows Archive,* 457: 77-85. doi:10.1007/s00428-010-0932-6

Jenkins, E. C., Debnath, S., Gundry, S., Gundry, S., Uyar, U., & Fata, J. E., (2012). Intracellular pH regulation by Na^+/H^+ exchanger-1 (NHE1) is required for growth factor-induced mammary branching morphogenesis. *Developmental Biology, 365*(1): 71-81. doi:10.1016/j.ydbio.2012.02.010

Jepsen, K. J., (2011). Functional interactions among morphologic and tissue quality traits define bone quality. *Clinical Orthopaedics and Related Research, 469*(8): 2150-2159. doi:10.1007/s11999-010-1706-9

Jiang, X., Iseki, S., Maxson, R. E., Sucov, H. M., & Morriss-Kay, G. M., (2002). Tissue origins and interactions in the mammalian skull vault. *Developmental Biology, 241*(1): 106-116. doi:10.1006/dbio.2001.0487

Jin, F., Jian, Y., Jian, C. & Jiahu, F. (2016). The role of chondrocytes in fracture healing. Journal of Spine 5(4): 1-8.

Joeng, S. K., & Long, F., (2014). Wnt7b can replace Ihh to induce hypertrophic cartilage vascularization but not osteoblast differentiation during endochondral bone development. *Nature* (*Bone Research),* 2: 14004.

Jorgensen, N. R., Teilmann, S. C., Henriksen, Z., Civitelli, R., Sorensen, O. H., & Steinberg, T. H., (2003). Activation of L-type calcium channels is required for gap junction-mediated intercellular calcium signaling in osteoblastic cells. *Journal of Biological Chemistry,* 278: 4082-4086.

Jos, V. S., Jones, D., Richards, R. G., Vico, L., Gasser, J. A., & Koller, B., (2005). Culture system for bone metabolic studies. *Microgravity Applications Programme*, 306-315.

Joyce, N. C., Hache, L. P., & Clemens, P. R., (2012). Bone health and associated metabolic complications in neuromuscular diseases. *Physical Medicine and Rehabilitation Clinics of North America,* 23(4): 773-779. doi:10.1016/j.pmr.2012.08.005

Jurdic, P., Saltel, F., Chabadel, A., & Destaing, O., 2006). Podosome and sealing zone : Specificity of the osteoclast model. *European Journal of Cell Biology, 85*: 195-202. doi:10.1016/j.ejcb.2005.09.008

Kanczler, J.M., Smith, E.L., Roberts, C.A., & Oreffo, R.O.C., (2012). A novel approach for studying the temporal modulation of embryonic skeletal development using organotypic bone cultures and micro-computed tomography. *Tissue Eng Part C Methods,* 18(10): 747–760.

Kant, S., Kumar, A., & Singh, S. M., (2014). Bicarbonate transport inhibitor SITS modulates pH homeostasis triggering apoptosis of Dalton's lymphoma: implication of novel molecular mechanisms. *Molecular and Cellular Biochemistry,* 167-178. doi:10.1007/s11010-014-2184-2

Karakas, E., & Furukawa, H., (2014). Crystal structure of a heterotetrameric NMDA receptor ion channel. *Science Magazine,* 344(6187): 992-997

Karmazyn, M., Sawyer, M., & Fliegel, L., (2005). The Na+/H+ exchanger: a target for cardiac therapeutic intervention. *Cardiovascular and Hematological Disorders-Drug Targets*, 5(4): 323-335.

Katagiri, T., & Takahashi, N., (2002). Bone biology regulatory mechanisms of osteoblast and osteoclast differentiation. *Oral Diseases* 8: 147-159.

Kidd, J. F., & Thorn, P., (2000). Intracellular Ca^{2+} and Cl^- channel activation in secretory cells. *Annual Reviews of Physiology,* 62: 493-513. DOI: 10.1146/annurev.physiol.62.1.493

Kim, D., Kim, J., Burghardt, B., Best, L., & Steward, M. C., (2014). Role of anion exchangers in Cl- and HCO3- secretion by the human airway epithelial cell line Calu-3. *American Journal of Physiology. Cell Physiology, 307*(2): C208-19. doi:10.1152/ajpcell.00083.2014

Kim, J. H., Liu, X., Wang, J., Chen, X., Zhang, H., Kim, S. H. & He, T.C., (2013). Wnt signaling in bone formation and its therapeutic potential for bone diseases. *Therapeutic Advances in Musculoskeletal Disease, 5*(1): 13-31. doi:10.1177/1759720X12466608

Kini, U., & Nandeesh, B. N., (2012). Physiology of bone formation, remodeling, and metabolism. In Forgelman, I., Gnanasegran, G. & van der wall, H. (Eds.), *Radinuclitide and Hybrid Bone Imaging*. (Vol. 1, 29-57): Springe.

Klein-Nulend, J., Bacabac, R. G., & Bakker, D., (2012). Mechanical loading and how it affects bone cells: The role of the osteocyte cytoskeleton in maintaining our skeleton. *European Cells and Materials, 24*: 278-291. doi:vol024a20 [pii]

Knothe Tate, M. L., Adamson, J. R., Tami, A. E., & Bauer, T. W., (2004). The osteocyte. *The International Journal of Biochemistry and Cell Biology, 36*(1): 1-8. doi:10.1016/S1357-2725(03)00241-3

Kobayashi, S., Takahashi, H., Ito, A., Saito, N., Nawata, M., Horiuchi, H., & Takaoka, K., (2003). Trabecular minimodeling in human iliac bone. *Bone, 32*(2): 163-169. doi:10.1016/S8756-3282(02)00947-X

Koester, K. J., Barth, H. D., & Ritchie, R. O., (2011). Effect of aging on the transverse toughness of human cortical bone: Evaluation by R-curves. *Journal of Mechanical Behavior of Biomedical Materials,* 4: 1 5 0 4-1 5 1 3. doi:10.1016/j.jmbbm.2011.05.020

Kogianni, G., & Noble, B., (2007). The biology of osteocytes. *Current Osteoporosis Reports, 5*(2): 81-86. doi:10.1007/s11914-007-0007-z

Kohler, M., Püschel, K., Sakharov, D., Tonevitskiy, A., Schänzer, W., & Thevis, M., (2008). Detection of recombinant growth hormone in human plasma by a 2-D PAGE Method. *Electrophoresis, 29*(22): 4495-4502. doi:10.1002/elps.200800221

Kojima, A., Toshima, J. Y., Kanno, C., Kawata, C., & Toshima, J., (2012). Localization and functional requirement of yeast Na+/H+ exchanger, Nhx1p, in the endocytic and protein recycling pathway. *Biochimica et Biophysica Acta,* 1823 (2): 534-543. doi: 10.1016/j.bbamcr.2011.12.004.

Komori, T., (2006). Regulation of osteoblast differentiation by transcription factors. *Journal of Cellular Biochemistry, 99*: 1233-1239. doi:10.1002/jcb.20958

Komori, T., (2013). Functions of the osteocyte network in the regulation of bone mass. *Cell and Tissue Research, 352*(2): 191-198. doi:10.1007/s00441-012-1546-x

Kooistra, B. W., Dijkman, B. G., Busse, J. W., prague, S., Schemitsch, E. H., & Bhandari, M., (2010): The radiographic union scale in tibial fractures: reliability and validity. *Journal of Orthopaedic and Traumatology,* 24: S81-S86.

Kotha, S. P., & Guzelsu, N., (2002). Modeling the tensile mechanical behaviour of bone along the longitudinal direction. *Journal of Theoretical Biology,* 219: 269-279.

Kratzel, C., Bergmann, C., Duda, G., Greiner, S., Schmidmaier, G., & Wildemann, B., (2008). Characterization of a rat osteotomy model with impaired healing. *BMC Musculoskeletal Disorders, 9*(1): 135. doi:10.1186/1471-2474-9-135

Kristensen, M., Hansen, T., & Juel, C., (2006). Membrane proteins involved in potassium shifts during muscle activity and fatigue. *American Journal of Physiology.Regulatory, Integrative and Comparative Physiology, 290*(3): R766-R7672.

Kronenberg, H. M., (2003). Developmental regulation of the growth plate. *Nature, International Weekly Journal of Science,* 423(6937): 332-336. doi:10.1038/nature01657

Kusuzaki, K., Kageyama, N., Shinjo, H., Takeshita, H., Murata, H., Hashiguchi, S., & Hirasawa, Y., (2000). Development of bone canaliculi during bone repair. *Bone, 27*(5): 655-9. doi:10.1016/S8756-3282(00)00383-5

Lakhkar, N. J., Lee, I. H., Kim, H. W., Salih, V., Wall, I. B., & Knowles, J. C., (2013). Bone formation controlled by biologically relevant inorganic ions: Role and controlled delivery from phosphate-based glasses. *Advanced Drug Delivery Reviews, 65*(4): 405-420. doi:10.1016/j.addr.2012.05.015

Lamraski, G., Monsaert, A., De Maeseneer, M., & Haentjens, P., (2006). Reliability and validity of plain radiographs to assess angulation of small finger metacarpal neck fractures: Human cadaveric study. *Journal of Orthopaedic Research, 24*(1): 37-45. doi:10.1002/jor.20025

Landowski, C. P., Suzuki, Y., & Hediger, M. A., (2012).The Mammalian transporter families. In A R. J., lpern, M. J., Caplan, & O. W., Moe, (Eds.), The Kidney Physiology and Pathophysiology (Vol. 5. 91-146). Elsevier.

Landry, P. S., Marino, A. A., Sadasivan, K. K., & Albright, J. A., (2000): Effect of soft-tissue trauma on the early periosteal response of bone to injury. *Journal of Trauma-Injury, Infection and Critical Care,* 48(3): 479-83.

Lane, M., Baltz, J. M., & Bavister, B. D., (1999). Bicarbonate/chloride exchange regulates intracellular pH of embryos but not oocytes of the hamster. *Biology of Reproduction,* 61: 452-457.

Lang, F., & Hoffmann, E. K., (2009). Cell volume regulation and cell survival. In: Lang, F. (Ed.) Mechanisms and significance of cell volume regulation. *Journal of the American College of Nutrition, 26*(5): 613S-623S.

LaStayo, P. C., Winters, K. M., & Hardy, M., (2003). Fracture healing: bone healing, fracture management, and current concepts related to the hand. *Journal of Hand Therapy, 16*(2): 81-93. doi:10.1016/S0894-1130(03)80003-0

Lauritzen, G., Stock, C. M., Lemaire, J., Lund, S. F., Jensen, M. F., Damsgaard, B., & Pedersen, S. F., (2012). The Na +/H + exchanger NHE1, but not the Na +, HCO3- cotransporter NBCn1, regulates motility of MCF7 breast cancer cells expressing constitutively active ErbB2. *Cancer Letters, 317*(2): 172-183. doi:10.1016/j.canlet.2011.11.023

Le, X., Miclau, T., Hu, D., & Helms, J., (2001). Molecular aspects of healing in stabilized and non-stabilized fractures. *Journal of Orthopaedic Research, 19*(1): 78-84. doi:10.1016/S0736-0266(00)00006-1

Lenoir, G., Williamson, P., & Holthuis, J. C. M., (2007). On the origin of lipid asymmetry: the flip side of ion transport. *Current Opinion in Chemical Biology, 11*(6): 654-61. doi:10.1016/j.cbpa.2007.09.008

Leung, K. C., Howe, C., Gui, L. Y., Trout, G., Veldhuis, J. D., & Ho, K. K. Y., (2002). Physiological and pharmacological regulation of 20-kDa growth hormone. *American Journal of Physiology. Endocrinology and Metabolism, 283*(4): E836–E843. doi:10.1152/ajpendo.00122.2002

Lewis, R., Asplin, K. E., Bruce, G., Dart, C., Mobasheri, A., & Barrett-Jolley, R., (2011a). The role of the membrane potential in chondrocyte volume regulation. *Journal of Cellular Physiology, 226*(11): 2979-2986. doi:10.1002/jcp.22646

Lewis, R., Feetham, C. H., & Barrett-Jolley, R., (2011b). Cell Volume Regulation in Chondrocytes. *Cellular Physiology and Biochemistry* 28: 1111-1122. DOI:10.1159/000335847

Lewis, R., Feetham, C.H., Gentles, L., Penny, J., Tregilgas, L., Tohami, W., Mobasheri, Ali. & Barrett-Jolley, R. (2013). Benzamil sensitive ion channels contribute to volume regulation in canine chondrocytes. *British Journal of Pharmacology* 168 (7): 1584-1596.

Li, H., Jiang, J., Wu, Y., & Chen, S. (2012). Potential mechanisms of a periosteum patch as an effective and favourable approach to enhance tendon-bone healing in the human body. *International Orthopaedics 36*(3): 665-669. doi:10.1007/s00264-011-1346-z

Li, H., Zhang, X., Wang, F., Li, R. & Wang, S. (2003): A Study on Proliferation of Chondrocyte in Callus during Second Fracture Healing. *Journal of Sichuan University (Medical Science edition)* 34(2): 274-276.

Li, J., Ahmad, T., Bergström, J., Samnegård, E., Erlandsson-Harris, H., Ahmed, M., & Kreicbergs, A., (2004). Differential bone turnover in an angulated fracture model in the rat. *Calcified Tissue International, 75*(1): 50-9. doi:10.1007/s00223-004-0206-x

Li, J., Ahmed, M., Samnegard, E., Ahmad, T., Stark, A., & Kreicbergs, A., (2005). Spontaneous correction of angular fracture deformity in the rat. *Acta Orthopaedica, 76*(3): 434–441. doi:10.1080/17453670510041358

Li, Z., Kong, K., & Qi, W., (2006). Osteoclast and its roles in calcium metabolism and bone development and remodeling. *Biochemical and Biophysical Research Communications,* 343: 345-350. doi:10.1016/j.bbrc.2006.02.147

Lindinger, M. I., Leung, M., Trajcevski, K. E., & Hawke, T. J., (2011). Volume regulation in mammalian skeletal muscle: The role of sodium-potassium-chloride cotransporters during exposure to hypertonic solutions. *The Journal of Physiology, 589*(Pt 11): 2887-2899.

Lindsay, R., Cosman, F., Zhou, H., Bostrom, M. P., Shen, V. W., Cruz, J. D., & Dempster, D. W., (2005). A novel tetracycline labeling schedule for longitudinal evaluation of the short-term effects of anabolic therapy with a single Iliac crest bone biopsy: early actions of teriparatide. *Journal of Bone and Mineral Research*, *21*(3): 366-373. doi:10.1359/JBMR.051109

Lindsey, A. E., Schneider, K., Simmonst, D. M., Baront, R. Lee, B. S., & Kopito, R. R., (1990). Functional expression and sub cellular localization of an anion exchanger cloned from choroid plexus. Cell Biology, Proceeding of National Academy of Science, 87: 5278-5282.

Lindsey, R. C., & Mohan, S., (2015). Skeletal effects of growth hormone and insulin-like growth factor-I therapy. *Molecular and Cellular Endocrinology*. Article in press. doi:10.1016/j.mce.2015.09.017

Lisignoli, G., Cristino, S., Piacentini, A., Toneguzzi, S., Grassi, F., Cavallo, C., & Facchini, A., (2005). Cellular and molecular events during chondrogenesis of human mesenchymal stromal cells grown in a three-dimensional hyaluronan based scaffold. *Biomaterials, 26*(28): 5677–5686. doi:10.1016/j.biomaterials.2005.02.031

Liu, C. J., Hwang, J. M., Wu, T. T., Hsieh, Y. H., Wu, C. C., Hsieh, Y. S., & Liu, J. Y., (2008). Anion exchanger inhibitor DIDS induces human poorly-differentiated malignant hepatocellular carcinoma HA22T cell apoptosis. *Molecular and Cellular Biochemistry 308*(1-2): 117–25. doi:10.1007/s11010-007-9619-y

Lokman, N. A., Elder, A. S. F., Ricciardelli, C. & Oehler, M. K. (2012). Chick chorioallantoic membrane (CAM) assay as an in vivo model to study the effect of newly identified molecules on ovarian cancer invasion and metastasis. *International Journal of Molecular Science* 13: 9959-9970.

Long, F. & Ornitz, D. M. (2013). Development of the endochondral skeleton. *Cold Spring Harbor Perspectives in Biology 5*(1): 1-20. doi:10.1101/cshperspect.a008334

Long, F., Chung, U., Ohba, S., McMahon, J., Kronenberg, H. M. & McMahon, A. P. (2004). Ihh signaling is directly required for the osteoblast lineage in the endochondral skeleton. *Development 131*: 1309-1318. doi:10.1242/dev.01006

Loqman, M. Y., Bush, P. G., Farquharson, C. & Hall, A. C. (2010). A cell shrinkage artefact in growth plate chondrocytes with common fixative solutions: importance of fixative osmolarity for maintaining morphology. *European Cells and Materials* 19: 214-224.

Loqman, M. Y., Bush, P. G., Farquharson, C. & Hall, A. C. (2013). Suppression of mammalian bone growth by membrane transport inhibitors. *Journal of Cellular Biochemistry 114*(3): 658-68. doi:10.1002/jcb.24408

Lorenzo, J. A., Holtrop, M. E. & Raisz, L. E. (1984). Effects of Phosphate on Calcium Release, Lysosomal Enzyme Activity in the Medium, and Osteoclast Morphometry in Cultured Fetal Rat Bones. *Bone* 5 (4): 187-190.

Lu, C., Miclau, T., Hu, D., & Marcucio, R. S. (2007). Ischemia leads to delayed union during fracture healing: a mouse model. *Journal of Orthopaedics Research* 25(1): 51-61. doi:10.1002/jor.20264.Ischemia

Lu, Y., Yeung, N., Sieracki, N. & Marshall, N. M. (2009). Design of functional metalloproteins. *Nature 460*(7257): 855-862. doi:10.1038/nature08304

Lynch, R. G. (2008). Tissue Culture of Mammalian Cells. *American Society for Investigative Pathology Pathway* 3(2): 31.

Lytle, C. & McManus, T. (2002). Coordinate modulation of Na-K-2Cl cotransport and K-Cl cotransport by cell volume and chloride. *American Journal of Physiology. Cell Physiology, 283*(5): C1422-C1431. doi:10.1152/ajpcell.00130.2002

MacDonald, T. L., Allen, D. A., & Monteith, G. J., (2013). Clinical assessment following tibial tuberosity advancement in 28 stifles at 6 months and 1 year after surgery. *Canadian Veterinary Journal, 54*(3): 249-254.

Mackie, E. J., Ahmed, Y., Tatarczuch, L., Chen, K. S., & Mirams, M., (2008). Endochondral ossification: How cartilage is converted into bone in the developing skeleton. *International Journal of Biochemistry and Cell Biology*, *40*(1): 46-62. doi:10.1016/j.biocel.2007.06.009

Mackie, E. J., Tatarczuch, L., & Mirams, M., (2011). The skeleton: a multi-functional complex organ: the growth plate chondrocyte and endochondral ossification. *Journal of Endocrinology,* 211:109-121.

Mackie, E. J., Tatarczuch, L., & Mirams, M., (2011). The skeleton: A multi-functional complex organ. The growth plate chondrocyte and endochondral ossification. *Journal of Endocrinology, 211*(2): 109-121. doi:10.1530/JOE-11-0048

Magne, D., Bluteau, G., Faucheux, C., Palmer, G., Vignes-Colombeix, C., Pilet, P., & Guicheux, J., (2003). Phosphate is a specific signal for ATDC5 chondrocyte maturation and apoptosis-associated mineralization: possible implication of apoptosis in the regulation of endochondral ossification. *Journal of Bone Mineral Research, 18*(8): 1430-1442. doi:10.1359/jbmr.2003.18.8.1430

Malo, M. E., & Fliegel, L., (2006). Physiological role and regulation of the Na+/H+ exchanger. *Canadian Journal of Physiology and Pharmacology, 84*: 1081-1095. doi:10.1139/y06-065

Malumbres, R., Lecanda, J., Melero, S., Ciesielczyk, P., Prieto, J., & Medina, J. F., (2003). HNF1α upregulates the human AE2 anion exchanger gene (SLC4A2) from an alternate promoter. *Biochemical and Biophysical Research Communications, 311*(1): 233-240. doi:10.1016/j.bbrc.2003.09.200

Manigrasso, M., & O'Connor, J., (2004). Characterization of a closed femur fracture model in mice. *Journal of Orthopaedic Trauma, 18*(10): 687-695. doi:00005131-200411000-00006

Manjunathan, R., & Ragunathan, M., (2015). Chicken chorioallantoic membrane as a reliable model to evaluate osteosarcoma- an experimental approach using SaOS2 cell line. *Biological Procedures Online,* 17: 10.

Marsell, R., & Einhorn, T., (2011). The biology of fracture healing. *Injury, 42*(6): 551-555. doi:10.1016/j.injury.2011.03.031

Mårtensson, K., Chrysis, D., & Sa¨vendahl, S., (2004). Interleukin-1β and TNF-α act in synergy to inhibit longitudinal growth in fetal rat metatarsal bones. *Journal of Bone Mineral Research,* 19: 1805-1812.

Martin, E. A., Ritman, E. L. & Turner, R. T. (2003). Time course of epiphyseal growth plate fusion in rat tibiae. *Bone,* 32: 261-267.

Martin, T. J., & Seeman, E., (2008). Bone remodelling: its local regulation and the emergence of bone fragility. *Best Practice in Research Clinical Endocrinology and Metabolism, 22*(5): 701-722. doi:10.1016/j.beem.2008.07.006

Marturano, J. E., Cleveland, B. C., Byrne, M. A., O'Connell, S. L., Wixted, J. J., & Billiar, K. L., (2008). An improved murine femur fracture device for bone healing studies. *Journal of Biomechanics, 41*(6): 1222-1228. doi:10.1016/j.jbiomech.2008.01.029

Masereel, B., (2003). An overview of inhibitors of Na+/H+ exchanger. *European Journal of Medicinal Chemistry, 38*(6): 547-554. doi:10.1016/S0223-5234(03)00100-4

Matos, M. A., Gonçalves, R. R., & Araújo, F. P., (2001): Experimental model for osteotomy in immature rabbit. *Acta Ortopédica Brasileira,* 9: 21-6. 10.1590/S1413-78522001000400003.

Mavčič, B., & Antolič, V., (2012). Optimal mechanical environment of the healing bone fracture/osteotomy. *International Orthopaedics, 36*(4): 689-695. doi:10.1007/s00264-012-1487-8

Maxhimer, J. B., James, P. B., & Justine, C. L., (2015). Signaling pathways in osteogenesis and osteoclastogenesis: Lessons from cranial sutures and applications to regenerative medicine. *Gene and Diseases,* 2 (1): 57-68. doi.org/10.1016/j.gendis.2014.12.004

McBratney-Owen, B., Iseki, S., Bamforth, S. D., Olsen, B. R., & Morriss-Kay, G. M., (2008). Development and tissue origins of the mammalian cranial base. *Developmental Biology, 322*: 121-132. doi:10.1016/j.ydbio.2008.07.016

MacDonald, T. L., Allen, D. A., & Monteith, G. J. (2013). Clinical assessment following tibial tuberosity advancement in 28 stifles at 6 months and 1 year after.

McKinney, M. C., & Kulesa, P. M., (2011). *In vivo* Calcium Dynamics during Neural Crest Cell Migration and Patterning using GCaMP3. *Developmental Biology,* 358: 309-317.

McNamara, L. M., Majeska, R. J., Weinbaum, S., Friedrich, V., & Schaffler, M. B., (2009). Attachment of osteocyte cell processes to the bone matrix. *Anatomy Record,* 292 (3): 355-363. doi:10.1002/ar.20869.

Medina, J. F., (2011). Role of the anion exchanger 2 in the pathogenesis and treatment of primary biliary cirrhosis. *Digestive Diseases, 29*(1): 103-12. doi:10.1159/000324144

Medina, J. F., Lecanda, J., Acín, a, Ciesielczyk, P., & Prieto, J., (2000). Tissue-specific N-terminal isoforms from overlapping alternate promoters of the human AE2 anion exchanger gene. *Biochemical and Biophysical Research Communications, 267*(1): 228-35. doi:10.1006/bbrc.1999.1951

Medina, J. F., Recalde, S., Prieto, J., Lecanda, J., Saez, E., Funk, C. D., & Elferink, R. P., J. O. (2003). Anion exchanger 2 is essential for spermiogenesis in mice. *Proceedings of the National Academy of Sciences of the United States of America, 100*(26): 15847-52. doi:10.1073/pnas.2536127100

Mehta, M., Schmidt-Bleek, K., Duda, G. N., & Mooney, D. J., (2012). Biomaterial delivery of morphogens to mimic the natural healing cascade in bone. *Advance Drugs Delivery Reviews,* 64(12):1257-76. doi:10.1016/ j.addr.2012.05.006

Meinhardt, U. J., & Ho, K. K., (2006). Modulation of growth hormone action by sex steroids. *Clinical Endocrinology,* 65 (4): 413-422. doi:10.1111/j.1365-2265.2006.02676.x

Melero-Martin, J. M., & Al-Rubeai, M., (2007). In vitro expansion of chondrocytes. In N., Ashammakhi, R., Reis, & E., Chiellini, (Eds.), *Topics in Tissue Engineering*, (Vol. 3. pp. 1-37). doi:10.1128/JVI.06620-11

Messer, H. H., (1977). Hormonal responses of bone in a continuous flow cultural system. *Journal of Dent Research,* 56(8): 971-975.

Mi, M., Jina, H., Wanga, B., Yukataa, K., Sheua, T., Kee, Q. H., Tongc, P., Ime, H., Xiaoe, G., & Chen, D., (2013). Chondrocyte BMP2 signaling plays an essential role in bone fracture healing. *Gene,* 512(2): 211-218. doi:10.1016/j.gene.2012.09.130.

Miękisz, J., Gomułkiewicz, J., & Miękisz, S., (2014). Mathematical models of ion transport through cell membrane channels. *Mathematica Applicanda, 42*(1): 39-62. doi:10.14708/ma.v42i1.469

Milan, J. L, Planell, J. A., & Lacroix, D., (2009). Computational modelling of the mechanical environment of osteogenesis within a polylactic acid-calcium phosphate glass scaffold. *Biomaterials,* 30: 4219-4226.

Mills, L., & Simpson, H. R. W., (2012). In vivo models of bone repair. *The Journal of Bone and Joint Surgery. British Volume, 94*(7): 865-74. doi:10.1302/0301-620X.94B7.27370

Mirhadi, S., Ashwood, N., & Karagkevrekis, B., (2013). Factors influencing fracture healing. *Trauma, 15*(2): 140-155. doi:10.1177/1460408613486571

Misof, B. M., Gamsjaeger, S., Cohen, A., Hofstetter, B., Roschger, P., Stein, E., & Klaushofer, K., (2012). Bone material properties in premenopausal women with idiopathic osteoporosis. *Journal of Bone and Mineral Research, 27*(12): 2551-2561. doi:10.1002/jbmr.1699

Mohamad, S. F., Shuid, A. N., Mohamed, N., Fadzilah, F. M., Mokhtar, S. A., Abdullah, S., & Soelaiman, I. N., (2012). The effects of alpha-tocopherol supplementation on fracture healing in a postmenopausal osteoporotic rat model. *Clinics, 67*(9): 1077-1085. doi:10.6061/clinics/2012(09)16

Morgan, E. F., De-Giacomo, A., & Gerstenfeld, L. C., (2014): Overview of fracture healing and its assessment. *Methods in Molecular Biology,* 1130: 13-31. doi:10.1007/978-1-62703-989-5_2.

Morshed, S., (2014). Current options for determining fracture Union. *Advances in Medicine, 2014*: 1–12. doi:10.1155/2014/708574

Nagchowdhuri, P. S., Andrews, K. N., Robart, S., & Capehart, A. A., (2012). Versican knockdown reduces interzone area during early stages of chick synovial joint development. *Anatomy Record,* (Hoboken) 295: 397-409.

Nakajima, F., Goto, O. A., Moriya, H., Ninomiya, Y., Einhorn, T. A. & Yamazaki, M. (2001). Spatial and temporal gene expression in chondrogenesis during fracture healing and the effects of basic fibroblast growth factor. *Journal of Orthopaedic Research,* 19: 935-944.

Nakamura, N., Tanaka, S., Teko, Y., Mitsui, K., & Kanazawa, H., (2005). Four Na+/H+ exchanger isoforms are distributed to Golgi and post-Golgi compartments and are involved in organelle pH regulation. *Journal of Biological Chemistry,* 280(2): 1561-1572. DOI 10.1074/jbc.M410041200

Nakashima, K., & De Crombrugghe, B., (2003). Transcriptional mechanisms in osteoblast differentiation and bone formation. *Trends in Genetics, 19*(8): 458-466. doi:10.1016/S0168-9525(03)00176-8

Nilsson, O., & Baron, J., (2004). Fundamental limits on longitudinal bone growth: growth plate senescence and epiphyseal fusion. *Trends in Endocrinology and Metabolism,* 15(8): 371-374.

Nilsson, O., Marino, R., De-Luca, F., Phillip, M. & Baron, J. (2005). Endocrine regulation of the growth plate. *Hormone Research,* 64:157-165.

Nindl, B. C., Hymer, W. C., Deaver, D. R., & Kraemer, W. J., (2001). Growth hormone pulsatility profile characteristics following acute heavy resistance exercise. *Journal of Applied Physiology,* 91 (1): 163-72.

Noble, B. S., & Reeve, J., (2000). Osteocyte function, osteocyte death and bone fracture resistance. *Molecular and Cellular Endocrinology, 159*: 7-13. doi:10.1016/S0303-7207(99)00174-4

Noble, B. S., (2008). The osteocyte lineage. *Archives of Biochemistry and Biophysics, 473*(2): 106-111. doi:10.1016/j.abb.2008.04.009

Nørrelund, H., (2005). The metabolic role of growth hormone in humans with particular reference to fasting. *Growth Hormone and IGF Research, 15*: 95-122. doi:10.1016/j.ghir.2005.02.005

Numata, M., & Orlowski, J., (2001). Molecular cloning and characterization of a novel (Na1,K1)/H1 exchanger localized to the trans-golgi network. *The Journal of Biological Chemistry,* 276(20): 17387-17394.

Nyberg, F., & Hallberg, M., (2013). Growth hormone and cognitive function. *Nature Review Endocrinology,* 9 (6): 357-65. doi:10.1038/nrendo.2013.78

O'Connor, R. D., Farach-Carson, M. C., & Schaven, N. C., (2010). Genetic and epigenetic aspect of bone development. In F., Bronner, M. C., Farach-Carson, & H. I., Roach, (Eds.). *Bone and Development, Topics in Bone Biology* (Vol. 6. pp. 1-24): Springer.

Ohlsson, C., Bengtsson, B. Å., Isaksson, O. G. P., Andreassen, T. T., & Slootweg, M. C., (1998). Growth hormone and bone. *Endocrine Reviews, 19*(1): 55-79. doi:10.1097/MED.0b013e3283319e6d

Okubo, N., Minam, Y., Fujiwara, H., Umemura, G. M. Tsuchiya, Y., Shirai, T., Oda, R., Arai, H., Inokawa, T., Kubo, K., & Yagita, K., (2013). Prolonged bioluminescence monitoring in mouse *ex vivo* bone culture revealed persistent circadian rhythms in articular cartilages and growth plates. *Plus One,* 8(11): e78306

Okubo, N., Minam, Y., Fujiwara, R., Oda, Y., Arai, T., Shirai, T., Kubo, K., & Yagita, K., (2014). Juvenile mouse femur grows in organ culture keeping normal circadian clock; establishment of a new *ex vivo* model system for investigating the cross-talk mechanisms between bone growth and circadian clock within Femur. *Orthopaedic Research Society Annual Meeting,* poster no. 1416.

Okumura, N., Imai, S., Toyoda, F., Isoya, E., Kumagai, K., Matsuura, H., & Matsusue, Y., (2009). Regulatory role of tyrosine phosphorylation in the swelling-activated chloride current in isolated rabbit articular chondrocytes. *Journal of Physiology,* 587 (15): 3761-3776 3761

Olney, R. C., (2003). Regulation of bone mass by growth hormone. *Medical and Pediatric Oncology,* 41(3): 228-34.

Olsen, B. R., (2006). Bone embryology. In Favus, M. J., (Eds.), *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism,* (Vol. 1. pp. 1-6): American Society for Bone and Mineral Research.

Orlov, S. N., Koltsova, S. V., Kapilevich, L. V., Gusakova, S. V., & Dulin, N. O., (2015). NKCC1 and NKCC2: The pathogenetic role of cation-chloride cotransporters in hypertension. *Genes and Diseases, 2*(2): 186-196. doi:10.1016/j.gendis.2015.02.007

Orlowski, J., & Grinstein, S., (2003). Molecular and functional diversity of mammalian Na+/H+ exchangers. In M. Karmazyn, M. Avkiran and L. Fliegel, (Eds.), The Na+/H+ Exchanger, from Molecular to Its Role in Disease, (Vol. 1. pp. 17-34), Kluwer Academic Publishers.

Ornitz, D., & Marie, P., (2002). FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease. *Genes and Development,* 1446-1465. doi:10.1101/gad.990702.ized

Ortega, N., Behonick, D. J., & Werb, Z., (2004). Matrix remodeling during endochondral ossification. *Trends in Cell Biology,* 14: 86-93.

Oryan, A., Monazzah, S., & Bigham-Sadegh, A., (2015). Bone injury and fracture healing biology. *Biomedical Environmental Science, 28*(1): 57-71. doi:10.3967/bes2015.006

Otto, T. E., Patke, P., & Haarman, H. J. Th., (1995). Closed fracture healing: a rat model. *European Surgical Research,* 27: 277-284.

Pastoureau, P. C., Hunziker, E. B., & Pelletier, J. P., (2010). Cartilage, bone and synovial histomorphometry in animal models of osteoarthritis. *Osteoarthritis and Cartilage, 18*: S106–S112. doi:10.1016/j.joca.2010.05.024

Pearce, A. I., Richards, R.G., Milz, S., Schneider, E., & Pearce, S G., (2010). Animal models for implant biomaterial research in bone: a review. *European Cells and Materials,* 13: 1-10.

Peavey, D. E., (2003). Endocrine regulation of calcium, phosphate and bone metebolism. In R. A., Rhodes, & G. A., Tanner, (Eds.), *Medical Physiology: Principles for Clinical Medicine*. (Vol. 1. pp. 634-648): Lippicott Williams and Wilkins.

Pfeilschifter, J., & Mundy, G. R., (1986). Modulation of type 13 transforming growth factor activity in bone cultures by osteotropic hormones. *Proceeding of National Academy of Science USA,* 84: 2024- 2028.

Pfeilschifter, S. M., Seyedin, T., & Mundy, G. R., (1988). Transforming growth factor beta Inhibits Bone Resorption in fetal rat Long Bone Cultures. *Journal of Clinical Investigation* 82: 680-685.

Phillips, M., (2005). Overview of the fracture healing cascade. *Injury*, *36 Suppl 3*: S5-S7. doi:10.1016/j.injury.2005.07.027

Pickering, S. W., & Scammell, B. E., (2002). Electromagnetic fields for bone healing. *The International Journal of Lower Extremity Wounds, 1*(3): 152-160. doi:10.1177/153473460200100302

Plotkin, L. I., Manolagas, S. C., & Bellido, T., (2002). Transduction of cell survival signals by connexin-43 hemichannels. *Journal of Biological Chemistry,* 277: 8648–8657.

Pontikoglou, C., Deschaseaux, F., Sensebé, L., & Papadaki, H., (2011). Bone marrow mesenchymal stem cells: biological properties and their role in hematopoiesis and hematopoietic stem cell transplantation. *Stem Cell Reviews, 7*(3): 569-89. doi:10.1007/s12015-011-9228-8

Porter, S. M., Dailey, H. L., Hollar, K. A., Klein, K., Harty, J. A., & Lujan, T. J., (2015). Automated measurement of fracture callus in radiographs using portable software. *Journal of Orthopaedic Research,* 34(1): doi:10.1002/jor.23146

Pound, J. C., Green, D. W., Chaudhuri, J. B., Roach, H. I., & Oreffo, R. O. C., (2006). Bioreactor culture of cartilage from mesenchymal populations. *Journal of Bone and Joint Surgery Britain,* 88: 405.

Pountos, I., Georgouli, T., Blokhuis, T. J., Pape, H. C., & Giannoudis, P. V., (2008). Pharmacological agents and impairment of fracture healing: What is the evidence? *International Journal of the Care of the Injured, 39*(4): 384-394. doi:10.1016/j.injury.2007.10.035

Pountos, I., Georgouli, T., Calori, G. M., & Giannoudis, P. V., (2012). Do nonsteroidal antiinflammatory drugs affect bone healing? A critical analysis. *The Scientific World Journal, 2012*, 606404. doi:10.1100/2012/606404

Purves, D., (2001). Channels and transporters. In D., Purves, G. J., Augustine, D., Fitzpatrick, L. C. Katz, A. S., LaMantia, J. O., McNamara, & S. M. Williams, (Eds.), *Neuroscience* (Vol. 2. pp. 20-48): Sinauer Associates Inc.

Raggatt, L. J., & Partridge, N. C., (2010). Cellular and molecular mechanisms of bone remodeling. *The Journal of Biological Chemistry,* 285 (33): 25103-25108.

Rahman, S., Naznin, A., Hossen, M. J., Rajat, S. B., & Sikder, M. A., (2015). TGF-β/BMP signaling and other molecular events: regulation of osteoblastogenesis and bone formation. *Bone Research,* 3: 15005. doi:10.1038/boneres.2015.5.

Raisz, L. G., & Nieman, I., (1969). Effect of phosphate, calcium and magnesium on bone resorption and hormonal Response in tissue culture. *Endocrinology,* 85: 446-452.

Raisz, L. G., (1965). Bone Resorption in tissue culture. Factors influencing the response to parathyroid hormone. *Journal of Clinical Investigation,* 44(1): 103-116.

Ranabir, S., & Reetu, K., (2011). Stress and hormones. *Indian Journal of Endocrinology and Metabolism,* 15 (1): 18-22. doi:10.4103/2230-8210.77573.

Rapaport, R., & Tuvemo, T., (2005). Growth and growth hormone in children born small for gestational age. *Acta Paediatrica, 94*(10): 1348–1355. doi:10.1080/08035250510043860

Reimold, F. R., Stewart, A. K., Stolpe, K., Heneghan, J. F., Shmukler, B. E., & Alper, S. L., (2013). Substitution of transmembrane domain Cys residues alters pHosensitive anion transport by AE2/SLC4A2 anion exchanger. *Pflugers Archiv,* 465(6): 839-851. doi:10.1007/s00424-012-1196-6.

Renshaw, S., (2007). Immunochemical staining techniques, In S., Renshaw (Ed.), *Immunohistochemistry: Methods Express*. (Vol. 1. pp. 45-96): Scion Publishing.

Rey, C., Combes, C., Drouet1, C., & Glimcher, M. J., (2010). Bone mineral: update on chemical composition and structure. *Osteoporosis International,* 20(6): 1013-1021. doi:10.1007/s00198-009-0860-y.Bone

Ribatti, D. Nico, B. Vacca, A., & Presta, M., (2006).The gelatin sponge-chorioallantoic membrane assay. *Nature Protocols,* 1(1): 85-91.

Ribatti, D., (2010). The Chick embryo chorioallantoic membrane as an *in vivo* assay to study antiangiogenesis. *Pharmaceuticals,* 3: 482-513.

Richards, R. G., Simpson, A. E., Jaehn, K., Furlong, P. I., & Stoddart, M. J., (2007). Establishing a 3D *ex vivo* culture system for investigations of bone metabolism and biomaterial interactions. *Alternative to Animal Experimentation,* 24: 56-59.

Rivas, R., & Shapiro, F., (2002). Structural stages in the development of the long bones and epiphyses: a study in the New Zealand white rabbit. *Joint and Bone Surgery America,* 84(1): 85-100.

Roach, H. I., (1990). Long-term organ culture of embryonic chick femora: A system for investigating bone and cartilage formation at an intermediate level of organization. *Journal of Bone Mineral Research,* 5: 85-100.

Roach, H. I., (1992a). Induction of normal and dystrophic mineralization by glycerophosphates in long-term bone organ culture. *Calcified Tissue International,* 50: 553-563.

Roach, H. I., (1992b). Trans-differentiation of hypertrophic chondrocytes into cells capable of producing a mineralized bone Matrix. *Bone Mineral Research,* 19: 1-20.

Roach, H. I., (1997). New aspects of endochondral ossification in the chick: chondrocyte apoptosis, bone formation by former chondrocytes, and acid phosphatase activity in the endochondral bone matrix. *Journal of Bone Mineral Research,* 12: 795-805.

Roach, H. I., Erenpreisa, J., & Aigner, T., (1995). Osteogenic differentiation of hypertrophic chondrocytes involves asymmetric cell divisions and apoptosis. *Journal of Cell Biology,* 131: 483-494.

Robey, P. G., & Boskey, A. L., (2006). Extracellular matrix and biomineralization of bone. In M. J., Favus. (Ed.), *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism,* (Vol. 1. pp. 12-19): American Society for Bone and Mineral Research

Rolian, C., (2008). Developmental basis of limb length in rodents: Evidence for multiple divisions of labor in mechanisms of endochondral bone growth. *Evolution and Development, 10*(1): 15-28. doi:10.1111/j.1525-142X.2008.00211.x

Romero, M. F., Chen, A., Parker, M. D., & Boron, W. F., (2013).The SLC4 family of bicarbonate (HCO3-) transporters. *Molecular Aspects of Medicine,* 34(2-3): 159-182. doi:10.1016/j.mam.2012.10.008.

Rossmann, H., Bachmann, O., Wang, Z., Shull, G. E., Obermaier, B., & Stuart-Tilley, U., (2001). Differential expression and regulation of AE2 anion exchanger subtypes in rabbit parietal and mucous cells. *The Journal of Physiology, 534*(Pt 3): 837-48. doi:PHY_12125

Rotter, R., Kraemer, R., Stratos, I., Vogt, P., Vollmar, B., Mittlmeier, T., & Knobloch, K., (2012). Compartmental and muscular response to closed soft tissue injury in rats investigated by oxygen-to-see and intravital fluorescence microscopy. *The Journal of Trauma and Acute Care Surgery, 73*(1): 73-9. doi:10.1097/TA.0b013e31824afddd

Rundle, C. H., Wang, X., Sheng, M. H. C., Wergedal, J. E., Lau, K. H. W., & Mohan, S., (2008). Bax deficiency in mice increases cartilage production during fracture repair through a mechanism involving increased chondrocyte proliferation without changes in apoptosis. *Bone, 43*(5): 880-888. doi:10.1016/j.bone.2008.07.239

Russell, J. M., (2000). Sodium-potassium-chloride cotransport. *Physiological Reviews, 80*(1): 211-276.

Saier, M. H., Reddy, V. S., Tamang, D. G., & Vastermark, A., (2014). The transporter classification database. *Nucleic Acids Research, 42*(D1): D251-D258. doi:10.1093/nar/gkt1097

Salem, A. K., Rose, F. R. A. J., Oreffo, R. O. C., Yang, X., Davies, M. C., Mitchell, J. R., Roberts, C. J., Stolnik-Trenkic, S., Tendler, S. J. B., Williams, P. M., & Shakesheff, K. M., (2003). Porous polymer and cell composites that self-assemble *in situ*. *Advance Materials,* 15: 210-213.

Saltel, F., Chabadel, A., Bonnelye, E., & Jurdic, P., (2008). Actin cytoskeletal organisation in osteoclasts: A model to decipher transmigration and matrix degradation. *European Journal of Cell Biology, 87*(8-9): 459-468. doi:10.1016/j.ejcb.2008.01.001

Sanchez, C. P., (2006). A dynamic bone revisited: is there progress? *Peritoneal Dialysis International : Journal of the International Society for Peritoneal Dialysis, 26*(1): 43-8.

Sánchez, J. C., Danks, T., & Wilkins, R. J., (2003). Mechanisms involved in the increase in intracellular calcium following hypotonic shock in bovine articular chondrocytes. *General Physiology and Biophysics, 22*: 487–500.

Sarangi, S., Mahapatra, a. P. K., Kundu, K., & Mohapatra, S., (2014). Functional biology of ion channels: a review. *Veterinary World, 7*(1): 13-16. doi:10.14202/vetworld.2014.13-16

Schaser, K. D., Bail, H. J., Schewior, L., Stover, J. F., Melcher, I., Haas, N. P., & Mittlmeier, T., (2005). Acute effects of N-acetylcysteine on skeletal muscle microcirculation following closed soft tissue trauma in rats. *Journal of Orthopaedic Research, 23*(1): 231-241. doi:10.1016/j.orthres.2004.05.009

Schelling, J. R., & Abu Jawdeh, B. G., (2008). Regulation of cell survival by Na/H exchanger-1. *American Journal of Physiology. Renal Physiology,* 295: F625-F632. doi:10.1152/ajprenal.90212.2008.

Schemitsch, E., & Kuzyk, P., (2009). The science of electrical stimulation therapy for fracture healing. *Indian Journal of Orthopaedics, 43*(2): 127. doi:10.4103/0019-5413.50846

Schindeler, A., McDonald, M. M., Bokko, P., & Little, D. G., (2008a). Bone remodeling during fracture repair: The cellular picture. *Seminars in Cell and Developmental Biology, 19*(5): 459-466. doi:10.1016/j.semcdb.2008.07.004

Schindeler, A., Morse, A., Harry, L., Godfrey, C., Mikulec, K., McDonald, M., & Little, D. G., (2008b). Models of tibial fracture healing in normal and Nf1-deficient mice. *Journal of Orthopaedic Research, 26*(8): 1053-60. doi:10.1002/jor.20628

Schmitz, N., Laverty, S., Kraus, V. B., & Aigner, T., (2010). Basic methods in histopathology of joint tissues. *Osteoarthritis and Cartilage, 18*: S113–S116. doi:10.1016/j.joca.2010.05.026

Schneider, P., Meier, M., Wepf, R., & Müller, R., (2010). Towards quantitative 3D imaging of the osteocyte lacuno-canalicular network. *Bone, 47*(5): 848-858. doi:10.1016/j.bone.2010.07.026

Schnell, D. J., & Hebert, D. N., (2003). Protein translocons: multifunctional mediators of protein translocation across membranes. *Cell, 112*(4): 491-505.

Schomann, T., Qunneis, F., Widera, D., Kaltschmidt, C., & Kaltschmidt, B., (2013). Improved method for ex ovo-cultivation of developing chicken embryos for human stem cell xenografts. *Stem Cells International,* 2013: 1-9.

Segev, O., Chumakov, I., Nevo, Z., Givol, D., Madar-Shapiro, L., Sheinin, Y., & Yayon, A., (2000). Restrained chondrocyte proliferation and maturation with abnormal growth plate vascularization and ossification in human FGFR-3G380R transgenic mice. *Human Molecular Genetics, 9*(2): 249-258.

Sfeir, C., Ho, L., Azari, K., & Hollinger, J., (2005). Fracture repair. In *Bone Regeneration and Repair: Biology and Clinical applications*. (Vol. 1. pp. 21-44): Springer doi:10.1385/1592598633

Shapiro, I. M., Adams, C. S., Freeman, T., & Srinivas, V., (2005). Fate of the hypertrophic chondrocyte: microenvironmental perspectives on apoptosis and survival in the epiphyseal growth plate. *Birth Defects Research (Part C),* 75:330-339. doi:10.1002/bdrc.20057

Shim, K. S. (2015). Pubertal growth and epiphyseal fusion. *Annals of Pediatric Endocrinology and Metabolism,* 20: 8-12

Shoji, T., Ii, M., Mifune, Y., Matsumoto, T., Kawamoto, A., Kwon, S., Kuroda, T., Kuroda, R., Kurosaka, M., & Asahara, T., (2010). Local transplantation of human multipotent adipose-derived stem cells accelerates fracture healing via enhanced osteogenesis and angiogenesis. *Laboratory Investigation,* 90: 637-649. doi: 10.1038/labinvest.2010.39.

Shum, L., Wang, X., Kane, A. A., & Nuckolls, G. H., (2003). BMP4 promotes chondrocyte proliferation and hypertrophy in the endochondral cranial base. *International Journal of Developmental Biology, 47*(6): 423-431.

Shum, L. & Nuckolls, G. (2002). The life cycle of chondrocytes in the developing skeleton. *Arthritis Research and Therapy,* 4: 94-106.

Sigurdsen, U. E. W., Reikeras, O., & Utvag, S. E., (2009). External fixation compared to intramedullary nailing of tibial fractures in the rat. *Acta Orthopaedica, 80*(3): 375-9. doi:10.3109/17453670903035567

Sims, N. A., & Martin, T. J., (2014). Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. *BoneKEy Reports3,* 481 (2014): 1-10. doi:10.1038/bonekey.2013.215

Sims, N. A., & Martin, T. J., (2015). Coupling Signals between the Osteoclast and Osteoblast: how are messages transmitted between these temporary visitors to the bone surface? *Frontiers in Endocrinology, 6*(41): 1-5. doi:10.3389/fendo.2015.00041

Singh, S., Wu, B. M., & Dunn, J. C., (2012). Delivery of VEGF using collagen-coated polycaprolactone scaffolds stimulates angiogenesis. *Journal of Biomedical Materials Research A,* 100: 720- 727

Slepkov, R., Rainey, J. K., Sykes, B. D., & Fliegel, L., (2007). Structural and functional analysis of the Na+/H+ exchanger family. *Biochemistry Journal,* 401: 623-633. doi:10.1042/BJ20061062

Smith, E. L, Rashidi, H., Kanczler, J. M., Shakesheff, K. M, & Oreffo, R. O. C., (2015). The effects of 1α, 25-dihydroxyvitamin D3 and transforming growth factor-β3 on bone development in an *ex vivo* organotypic culture system of embryonic chick femora. *PLoS ONE,* 10(4): e0121653.

Smith, E. L., Kanczler, J. M., & Oreffo, R. O. C., (2013). A new take on an old story: chick limb organ culture for skeletal niche development and regenerative medicine evaluation. *European Cells and Materials,* 26: 91-106.

Smith, E. L., Kanczler, M. J., Gothard, D., Roberts, C. A., Wells, A. J., White, L. J. Qutachi, O., Sawkins, M. J., Peto, H. Rashidi, H., Rojo, H., Stevens, M. M., El Haj, A. J., Rose, F. R. A.J., Shakesheff, K. M., & Oreffo, R. O. C., (2014). Evaluation of skeletal tissue repair, part 1: assessment of novel growth-factor-releasing hydrogels in an *ex vivo* chick femur defect model. *Acta Biomaterialia,* 10: 4186-4196.

Smith, E. L., Locke, M., Waddington, R. J., & Sloan, A. J., (2010). An *ex vivo* rodent mandible culture model for bone repair tissue. *Engineering Part C: Methods*, 16(6): 1287-1296. doi:10.1089/ten.tec.2009.0698.

Smith-Adaline, E. A., Volkman, S. K., Ignelzi, M. A., Slade, J., Platte, S., & Goldstein, S. A., (2004). Mechanical environment alters tissue formation patterns during fracture repair. *Journal of Orthopaedic Research, 22*(5): 1079-1085. doi:10.1016/j.orthres.2004.02.007

Soleimani, M., & Nadri, S., (2009). A protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow. *Nature Protocols, 4*(1): 102-106. doi:10.1038/nprot.2008.221

Spinardi, L., & Carlo, P., (2006). Podosomes as smart regulators of cellular adhesion. *European Journal of Cell Biology, 85*: 191-194. doi:10.1016/j.ejcb.2005.08.005

Srinivas, V. & Shapiro, I. M. (2012). The epiphyseal growth plate: The engine that drives bone elongation. In: Preedy, V. R. (Ed.) handbook of growth and growth monitoring in health and disease. Springer. Pp. 1331-1349.

Staines, K. A., Pollard, A. S., McGonnell, I. M., Farquharson, C., & Pitsillides, A. A., (2013). Cartilage to bone transitions in health and disease. *Journal of Endocrinology, 219*(1): R1–R12. doi:10.1530/JOE-13-0276

Staub, O., & Rotin, D., (2006). Role of ubiquitylation in cellular membrane transport. *Physiological Reviews, 86*(2): 669-707. doi:10.1152/physrev.00020.2005

Stern, P. H., & Krieger, N. S., (1983). Comparison of fetal rat Limb bones and neonatal mouse calvaria: effects of parathyroid hormone and 1, 25-Dihydroxyvitamin D3. *Calcified Tissue International,* 35: 172-176.

Studer, D., Millan, C., Öztürk, E., Maniura-Weber, K., & Zenobi-Wong, M., (2012). Molecular and biophysical mechanisms regulating hypertrophic differentiation in chondrocytes and mesenchymal stem cells. *European Cells and Materials, 24*: 118-135.

Suchý, P., Straková, E., Herzig, I., Steinhauser, L., Králik, G., & Zapletal, D., (2009). Chemical composition of bone tissue in broiler chickens intended for slaughter. *Czech Journal of Animal Science, 54*(7): 324-330.

Sugiura, J., Ito, H., & Sakurai, Y., (2006). Vascular invasion of epiphyseal growth plate in osteopetrotic (op/op) mouse tibiae. *Journal of Hard Tissue Biology, 15*(3): 96-100.

Sugiyama, T., Price, J. S., & Lanyon, L. E., (2010). Functional adaptation to mechanical loading in both cortical and cancellous bone is controlled locally and is confined to the loaded bones. *Bone, 46*(2): 314–321. doi:10.1016/j.bone.2009.08.054

Summerlee, A. J. S., (2001). Bone formation and development. In G., Summer-Smith, (Ed.), *Bone in Clinical Orthopaedics*. (Vol. 2, pp. 1-23): AO Publishing.

Sun, H., Zang, W., Zhou, B., Xu, L., & Wu, S., (2011). DHEA suppresses longitudinal Bone growth by acting directly at growth plate through estrogen receptors. *Endocrinology,* 152: 1423-1433.

Sun, M. M. G., & Beier, F., (2014). Chondrocyte hypertrophy in skeletal development, growth, and disease. *Birth Defects Research Part C - Embryo Today: Reviews*, *102*(1): 74-82. doi:10.1002/bdrc.21062

Sylvestre, A., Wilson, J., & Hare, J., (2002). A comparison of 2 different suture patterns for skin closure of canine ovariohysterectomy. *Canadian Veterinary Journal, 43: 699-702.*

Sys, G., Van Bockstal, M., Forsyth, R., Balke, M., Poffyn, B., Uyttendaele, D., Bracke, M., & De Wever, O., (2012). Tumor grafts derived from sarcoma patients retain tumor morphology, viability, and invasion potential and indicate disease outcomes in the chick chorioallantoic membrane model. *Cancer Letter,* 326: 69-78.

Taichman, R. S., (2005). Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. *Blood, 105*(7): 2631-269. DOI 10.1182/blood-2004-06- 2480.

Takata, K., Matsuzaki, T., & Tajika, Y., (2004). Aquaporins: water channel proteins of the cell membrane. *Progress in Histochemistry and Cytochemistry, 39*(1): 1-83.

Takeda, A., Cooper, K., Bird, a., Baxter, L., Frampton, G. K., Gospodarevskaya, E., & Bryant, J., (2010). Recombinant human growth hormone for the treatment of growth disorders in children: A systematic review and economic evaluation. *Health Technology Assessment, 14*(42): 1-237. doi:10.3310/hta14420

Takeda, T., Narita, T., & Ito, H., (2004). Experimental study on the effect of mechanical stimulation on the early stage of fracture healing. *Journal of Nippon Medical School, 71*(4): 252-262.

Tawonsawatruk, T., Hamilton, D. F., & Simpson, A. H. R. W., (2014). Validation of the use of radiographic fracture-healing scores in a small animal model. *Journal of Orthopaedic Research,* 32: 1117-1119.

Teitelbaum, S. L., & Ross, F. P., (2003). Genetic regulation of osteoclast development and function. *Nature Reviews Genetics,* 4: 638-649. doi:10.1038/nrg1122

Teng, G. Y. Y., & Liou, E. J. W., (2014). Interdental osteotomies induce regional acceleratory phenomenon and accelerate orthodontic tooth movement. *Journal of Oral and Maxillofacial Surgery, 72*(1): 19-29. doi:10.1016/j.joms.2013.09.012

Teti, A., (2013). Mechanisms of osteoclast-dependent bone formation. *BoneKEy Reports 4(*2): 449. doi:10.1038/bonekey.2013.183

Tivesten, A., Movérare-Skrtic, S., Chagin, A., Venken, K., Salmon, P., Vanderschueren, D., & Ohlsson, C., (2004). Additive protective effects of estrogen and androgen treatment on trabecular bone in ovariectomized rats. *Journal of Bone and Mineral Research, 19*(11): 1833-1839. doi:10.1359/JBMR.040819

Toppe, J., Albrektsen, S., Hope, B., & Aksnes, A., (2007). Chemical composition, mineral content and amino acid and lipid profiles in bones from various fish species. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 146*(3): 395-401. doi:10.1016/j.cbpb.2006.11.020

Tsang, K.Y., Chan, D. & Cheah, K.S.E. (2015). Fate of growth plate hypertrophic chondrocytes: Death or lineage extension? *Development Growth and Differenciation* 57: 179-192.

Tsangari, H., Kuliwaba, J. S., & Fazzalari, N. L., (2007). Trabecular bone modeling and subcapital femoral fracture. *Journal of Musculoskeletal and Neuronal Interaction,* 7(1): 69-73.

Tsiridis, E., Upadhyay, N., & Giannoudis, P., (2007). Molecular aspects of fracture healing: which are the important molecules? *Injury, 38*(1): S11–S25. doi:10.1016/j.injury.2007.02.006

Tsubota, K., & Adachi, T., (2005). Spatial and temporal regulation of cancellous bone structure: characterization of a rate equation of trabecular surface remodeling. *Medical Engineering and Physics,* 27: 305-311.

Turk, C., Halici, M., Guney, A., Akgun, H., Sahin, V., & Muhtaroglu, S., (2004). Promotion of fracture healing by vitamin E in rats. *The Journal of International Medical Research, 32*(5): 507-512. doi:10.1177/147323000403200508

Ubara, Y., Fushimi, T., Tagami, T., Sawa, N., Hoshino, J., Yokota, M., & Hara, S., (2003). Histomorphometric features of bone in patients with primary and secondary hypoparathyroidism. *Kidney International, 63*(5): 1809-1816. doi:10.1046/j.1523-1755.2003.00916.x

Ubara, Y., Tagami, T., Nakanishi, S., Sawa, N., Hoshino, J., Suwabe, T., & Takaichi, K., (2005). Significance of minimodeling in dialysis patients with adynamic bone disease. *Kidney International, 68*(2): 833-9. doi:10.1111/j.1523-1755.2005.00464.x

Ulstrup, K. A., (2008). Biomechanical concepts of fracture healing in weight-bearing long bones. *Acta Orthopaedica Belgica, 74*(3): 291-302.

Väänänen, H. K., & Laitala-leinonen, T., (2008). Osteoclast lineage and function. *Archives of Biochemistry and Biophysics, 473*: 132-138. doi:10.1016/j.abb.2008.03.037

Väänänen, H. K., Zhao, H., Mulari, M., & Halleen, J. M., (2000). The cell biology of osteoclast function. *Journal of Cell Science,* 113*:* 377-381.

Väänänen, K., (2005). Mechanism of osteoclast mediated bone resorption-rationale for the design of new therapeutics. *Advanced Drug Delivery Reviews, 57*: 959-971. doi:10.1016/j.addr.2004.12.018

Valles, P. G., Bocanegra, V., Gil Lorenzo, A., & Costantino, V. V., (2015). Physiological functions and regulation of the Na^+/H^+ exchanger [NHE1] in renal tubule epithelial cells. *Kidney and Blood Pressure Research,* 40(5): 452-466. doi:10.1159/000368521

Van Cauter, E., Latta, F., Nedeltcheva, A., Spiegel, K., Leproult, R., Vandenbril, C., Weiss, R., Mockel, J., Legros, J. J., & Copinschi, G., (2004). Reciprocal interactions between the GH axis and sleep. *Growth Hormone and IGF Research,* 14 Suppl A: S10-S17.doi:10.1016/j.ghir.2004.03.006.

van der Eerden, B. C. J., (2003). Systemic and local regulation of the growth plate. *Endocrine Reviews, 24*(6): 782–801. doi:10.1210/er.2002-0033

Vento, P. J., Swartz, M. E., Martin, L. B. E. & Daniels, D. (2008). Food Intake in Laboratory Rats Provided Standard and Fenbendazole-supplemented Diets. *Journal of the American Association for Laboratory Animal Science* 47(6): 46-50.

Vertenten, G., Gasthuys, F., Cornelissen, M., Schacht, E., & Vlaminck, L., (2010). Enhancing bone healing and regeneration: present and future perspectives in veterinary orthopaedics. *Veterinary and Comparative Orthopaedics and Traumatology,* 153-162. doi:10.3415/VCOT-09-03-0038

Victoria, G., Petrisor, B., Drew, B., & Dick, D., (2009). Bone stimulation for fracture healing: What's all the fuss? *Indian Journal of Orthopaedics, 43*(2): 117. doi:10.4103/0019-5413.50844

Villemure, I. & Stokes, I. A. F. (2009). Growth plate mechanics and mechanobiology. A survey of present understanding. *Journal of Biomechanics,* 42: 1793-1803

Vivanco, J., Garcia, S., Ploeg, H. L., Alvarez, G., Cullen, D., & Smith, E. L., (2013). Apparent elastic modulus of *ex vivo* trabecular bovine bone increases with dynamic loading. *Journal of Engineering in Medicine,* 227(8): 904-912.

von Pfeil, D. J. F., & DeCamp, C. E., (2009).The epiphyseal plate: physiology, anatomy and trauma. *Compendium of Continuing Education for Veterinarian,* (CE Article) 1-12.

Wada, T., Nakashima, T., Hiroshi, N., & Penninger, J. M., (2006). RANKL – RANK signaling in osteoclastogenesis and bone disease. *Trends in Molecular Medicine, 12*(1). doi:10.1016/j.molmed.2005.11.00

Waldron, K. J., Rutherford, J. C., Ford, D., & Robinson, N. J., (2009). Metalloproteins and metal sensing. *Nature, 460*(7257): 823-830. doi:10.1038/nature08300

Wang, W., Lian, N., Li, L., Moss, H. E., Wang, W., Perrien, D. S., & Yang, X., (2009). Atf4 regulates chondrocyte proliferation and differentiation during endochondral ossification by activating Ihh transcription. *Development, 136*(24): 4143-4153. doi:10.1242/dev.043281

Wang, Z., Schultheis, P. J., & Shull, G. E., (1996). Three N-terminal variants of the AE2 Cl2/HCO3 2 exchanger are encoded by mRNAs transcribed from alternative promoters. *The Journal of Biological Chemistry,* 271(13): 7835-7843.

Warden, S. J., (2006). Breaking the rules for bone adaptation to mechanical loading. *Journal of Applied Physiology, 100*(5): 1441-1442. doi:10.1152/japplphysiol.00038.2006

Warden, S. J., Komatsu, D. E., Rydberg, J., Bond, J. L., & Hassett, S. M., (2009). Recombinant human parathyroid hormone (PTH 1-34) and low-intensity pulsed ultrasound have contrasting additive effects during fracture healing. *Bone,* 44: 485-494

Webster, D. J., Schneider, P., Dallas, S. L., & Müller, R., (2013). Studying osteocytes within their environment. *Bone 54*(2): 285-295. doi:10.1016/j.bone.2013.01.004

Weiler, A., Helling, H. J., Kirch, U., Zirbes, T. K., & Rehm, K. E., (1996). Foreign-body reaction and the course of osteolysis after polyglycolide implants for fracture fixation: experimental study in sheep. *The Journal of Bone and Joint Surgery. British Volume, 78*(3): 369-376

Weise, M., De-Levi, S., Barnes, K. M., Gafni, R. I., Abad, V., & Baron, J., (2001). Effects of estrogen on growth plate senescence and epiphyseal fusion. *Proceedings of the National Academy of Sciences of the USA, 98*(12): 6871-6876. doi:10.1073/pnas.121180498

Wiebe, C., Dibattista, E. R., & Fliegel, L., (2001). Functional role of polar amino acid residues in Na+/H+ exchangers. *The Biochemical Journal, 357*(Pt 1): 1-10. doi:10.1042/0264-6021:3570001

Wildemann, B., Schmidmaier, G., Ordel, S., Stange, R., Haas, N. P., & Raschke, M., (2003). Cell proliferation and differentiation during fracture healing are influenced by locally applied IGF-I and TGF-beta1: comparison of two proliferation markers, PCNA and BrdU. *Journal of Biomedical Materials Research. Part B, Applied Biomaterials, 65*(1): 150-6. doi:10.1002/jbm.b.10512

Wilkins, R. J., Browning, J., & Ellory, J. C., (2000). Surviving in a matrix: membrane transport in articular chondrocytes. *The Journal of Membrane Biology, 177*(2): 95-108. doi:10.1007/s002320001103

Willey, J. S., Grilly, L. G., Howard, S. H., Pecaut, M. J., Obenaus, A., Gridley, D. S., & Bateman, T. A., (2008). Bone architectural and structural properties after 56Fe26+ radiation-induced changes in body mass. *Radiation Research, 170*(2): 201-207. doi:RR0832 [pii]\r10.1667/RR0832.1

Williams, J. L., Do, P. D., Eick, J. D. & Schmidt, T. L., 2001: Tensile properties of the physis vary with anatomic location, thickness, strain rate and age. *Journal of Orthopaedic Research,* 19 (6): 1043-1048.

Wilsman, N. J., Farnum, C. E., Green, E. M. Lieferman, E. M. & Clayto, M. K. (1996). Cell cycle analysis of proliferative zone chondrocytes in growth plates elongating at different rates. *Journal of Orthopaedics Research,* 14(4): 562-572

Williams, R. M., Zipfel, W. R., Tinsley, M. L., & Farnum, C. E., (2007). Solute transport in growth plate cartilage: in vitro and in vivo. *Biophysical Journal, 93*(3): 1039-50. doi:10.1529/biophysj.106.097675

Willie, B. M., Petersen, A., Schmidt-Bleek, K., Cipitria, A., Mehta, M., Strube, .P, Lienau, J., Wildemann, B.,Fratzl, P., & Duda, G., (2010). Designing biomimetic scaffolds for bone regeneration: why aim for a copy of mature tissue properties if nature uses a different approach? *Soft Matter,* 6: 4976-4987. DOI: 10.1039/c0sm00262c

Willie, B., Adkins, K., Zheng, X., Simon, U., & Claes, L., (2009). Mechanical characterization of external fixator stiffness for a rat femoral fracture model. *Journal of Orthopaedic Research, 27*(5): 687-693. doi:10.1002/jor.20792

Wilsman, N. J, Bernardini, E. S., Leiferman, E., Noonan, K., & Farnum, C. E., (2008). Age and pattern of the onset of differential growth among growth plates in rats. J*ournal of Orthopedic Research,* 26: 1457-1465. doi: 10.1002/jor.20547

Wit, J. M., & Camacho-Hübner, C., (2011). Endocrine regulation of longitudinal bone growth. *Endocrine Development, 21*: 30-41. doi:10.1159/000328119

Woods, A., Wang, G., & Beier, F., (2007). Regulation of chondrocyte differentiation by the actin cytoskeleton and adhesive interactions. *Journal of Cell Physiology,* 213(1): 1-8.

Wu, J., Glimcher, L. H., & Aliprantis, A. O., (2008). HCO3-/Cl- anion exchanger SLC4A2 is required for proper osteoclast differentiation and function. *Proceedings of the National Academy of Sciences of the USA, 105*: 16934-16939. doi:10.1073/pnas.0808763105

Wu, M., Li, Y. P., Zhu, G., Lu, Y., Wang, Y., Jules, J., & Chen, W., (2014). Chondrocyte-specific knockout of Cbfβ reveals the indispensable function of Cbfβ in chondrocyte maturation, growth plate development and trabecular bone formation in mice. *International Journal of Biological Sciences, 10*(8): 861-872. doi:10.7150/ijbs.8521

Wu, S., & De Luca, F., (2004). Role of Cholesterol in the regulation of growth plate chondrogenesis and longitudinal bone growth. *The Journal of Biological Chemistry,* 279 (6): 4642-4647.

Wulff, H., (2008). New light on the “old” chloride channel blocker DIDS. *ACS Chemical Biology, 3*(7): 399-401. doi:10.1021/cb800140m

Xing, L., & Boyce, B. F., (2005). Regulation of apoptosis in osteoclasts and osteoblastic cells. *Biochemical and Biophysics Research Communication,* 328: 709-720.

Xu, Y., Ramu, Y., & Lu, Z., (2008). Removal of phospho-head groups of membrane lipids immobilizes voltage sensors of K+ channels. *Nature, 451*(7180): 826-9. doi:10.1038/nature06618

Yalcin, H.C., Shekhar, A., Rane, A. A., & Butcher J.T., (2010). An ex-ovo chicken embryo Culture system suitable for imaging and microsurgery applications. *Journal of Visualized Experiment,* 44: DOI: 10.3791/2154

Yang, L., Tsang, K. Y., Tang, H. C., Chan, D., & Cheah, K. S. E., (2014). Hypertrophic chondrocytes can become osteoblasts and osteocytes in endochondral bone formation. *Proceedings of the National Academy of Sciences of the USA*, 111(33): 12097-12102. doi:10.1073/pnas.1302703111

Yang, X., & Karsenty, G., (2002). Transcription factors in bone: developmental and pathological aspects. *Trends in Molecular Medicine, 8*(7): 340-345. doi:10.1016/S1471-4914(02)02340-7

Yang, X., Wang, D., Dong, W., Song, Z., & Dou, K., (2010). Inhibition of Na+/H+ exchanger 1 by 5-(N-ethyl-N-isopropyl) amiloride reduces hypoxia-induced hepatocellular carcinoma invasion and motility. *Cancer Letters, 295*(2): 198-204. doi:10.1016/j.canlet.2010.03.001

Yang, X., Whitaker, M., Sebald, W., Clarke, N., Howdle, S., Shakesheff, K., & Oreffo, R., (2004). Human Osteoprogenitor bone formation using encapsulated bone morphogenetic protein 2 in porous polymer scaffolds. *Tissue Engineering,* 10: 1037-1045.

Yanga, L., Tsanga, K. Y., Tanga, H. C., Chana, D., & Kathryn S. E. C., (2014). Hypertrophic chondrocytes can become osteoblasts and osteocytes in endochondral bone formation. *Proceedings of the National Academy of Sciences of USA,* 111(33): 2097-2102.

Yavropoulou, M. P., & Yovos, J. G., (2008). Osteoclastogenesis current knowledge and future perspectives. *Journal of Musculoskeletal and Neuronal Interactions,* 8: 204-216.

Yerramshetty, J. S., & Akkus, O., (2008). The associations between mineral crystallinity and the mechanical properties of human cortical bone. *Bone,* 42: 476-482.

Yi, S., Bernat, B., Pál, G., Kossiakoff, A., & Li, W. H., (2002). Functional promiscuity of squirrel monkey growth hormone receptor toward both primate and nonprimate growth hormones. *Molecular Biology Evolution,* 19 (7): 1083-92. doi:10.1093/oxfordjournals.molbev.a004166.

Yool, A. J., & Campbell, E. M., (2012). Structure, function and translational relevance of aquaporin dual water and ion channels. *Molecular Aspects of Medicine, 33*(5-6): 553-561. doi:10.1016/j.mam.2012.02.001

Yoshida, T., Vivatbutsiri, P., Morriss-Kay, G., Saga, Y., & Iseki, S., (2008). Cell lineage in mammalian craniofacial mesenchyme. *Mechanisms of Development, 125*(9-10): 797-808. doi:10.1016/j.mod.2008.06.007

Young, M. T., & Tanner, M. J. A., (2003). Distinct regions of human glycophorin A enhance human red cell anion exchanger (Band 3; AE1) transport function and surface trafficking. *Journal of Biological Chemistry*, 278: 32954-32961.

Yu, L., & Hales, C. A., (2011). Silencing of sodium-hydrogen exchanger 1 attenuates the proliferation, hypertrophy, and migration of pulmonary artery smooth muscle cells via E2F1. *American Journal of Respiratory Cell and Molecular Biology,* 45(5): 923-930.

Yu, X., Botchwey, E. A., Levine, E. M., Pollack, S. R., & Laurencin, C. T., (2004). Bioreactor-based bone tissue engineering: The influence of dynamic flow on osteoblast phenotypic expression and matrix mineralization. *Proceeding of National Academy of Science* of the USA, 101: 11203-11208.

Zabielska, K., Lechowski, R., Krol, M., Pawlowski, K. M., Motyl, T., Dolka, I., & Zbikowski, A., (2012). Derivation of feline vaccine-associated fibrosarcoma cell line and Its growth on chick embryo chorioallantoic membrane- A new *in vivo* model for veterinary oncological studies. *Veterinary Research Communication,* 36: 227-233.

Zanelli, J. M., Lea, D. J., & Nisbel, J. A., (1969). Bioassay methods in vitro for parathyroid hormone. *Journal of Endocrinology,* 43: 33-46.

Zhang, F., He, Q., Tsang, W. P., Garvey, W. T., Chan, W. Y., & Wan, C., (2014). Insulin exerts direct, IGF-1 independent actions in growth plate chondrocytes. *Bone Research,* 2014(2): 14012. doi:10.1038/boneres.2014.12

Zhang, F., Xu, L., Xu, L., Xu, Q., Li, D., Yang, Y., & Chen, C. D., (2015). JMJD3 promotes chondrocyte proliferation and hypertrophy during endochondral bone formation in mice. *Journal of Molecular Cell Biology, 7*(1): 23-34. doi:10.1093/jmcb/mjv003

Zhang, X., Awad, H. A., O'Keefe, R. J., Guldberg, R. E., & Schwarz, E. M., (2008). A perspective: Engineering periosteum for structural bone graft healing. *Clinical Orthopaedics and Related Research, 466*(8): 1777-1787. doi:10.1007/s11999-008-0312-6

Zhang, Y., Dilaware, K., Julia, D., & Edda, T., (2012). Mechanisms underlying the osteo- and adipo-differentiation of human mesenchymal stem cells. *The Scientific World Journa,l* 2012: 1–14. doi:10.1100/2012/793823.

Zhao, H., Wiederkehr, M. R., Fan, L., Collazo, R. L., Crowder, L. A., & Moe, O. W., (1999). Acute inhibition of Na/H exchanger NHE-3 by cAMP. Role of protein kinase A and NHE-3 phosphoserines 552 and 605. *Journal of Biological Chemistry, 274*(7): 3978-3987.

Zhao, J., Zhao, X., Jiang, Z., Li, Z., Fan, X., Zhu, J., & Shi, J., (2014). Biomimetic and bioinspired membranes: Preparation and application. *Progress in Polymer Science, 39*(9): 1668-1720. doi:10.1016/j.progpolymsci.2014.06.001

Zhou, G., Zheng, Q., Engin, F., Munivez, E., Chen, Y., Sebald , E., Krakow, D., & Lee, B., (2006). Dominance of SOX9 function over RUNX2 during skeletogenesis. *Proceeding of National Academy of Science of USA,* 103(50): 19004-19009. doi: 10.1073/pnas.0605170103

Zhou, X., von der Mark, K., Henry, S., Norton, W., Adams, H., & de Crombrugghe, B., (2014). Chondrocytes transdifferentiate into osteoblasts in endochondral bone during development, postnatal growth and fracture healing in mice. *PLoS Genetics*, *10*(12): e1004820. doi:10.1371/journal.pgen.1004820

Zhu, Q., Lee, D. W. K., & Casey, J. R., (2003). Novel topology in C-terminal region of the human plasma membrane anion exchanger, AE1. *Journal of Biological Chemistry, 278*(5): 3112-3120. doi:10.1074/jbc.M207797200

Zhu, Z. H., Gao, Y. S., Luo, S. H., Zeng, B. F., & Zhang, C. Q., (2011). An animal model of femoral head osteonecrosis induced by a single injection of absolute alcohol: an experimental study. *Medical Science Monitor : International Medical Journal of Experimental and Clinical Research, 17*(4): BR97–102.

Zuo, G., Zhang, L., Qi, J., Kang, H., Jia, P., Chen, H., & Deng, L., (2015). Activation of HIFa pathway in mature osteoblasts disrupts the integrity of the osteocyte/canalicular network. *Plos One*, *10*(3): e0121266. doi:10.1371/journal.pone.012126