

The Mechanical Environment of Bone Marrow: A Review

UMUT ATAKAN GURKAN and OZAN AKKUS

Weldon School of Biomedical Engineering, Purdue University, 206 S. Martin Jischke Drive, West Lafayette, IN 47907-2032, USA

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Abstract—Bone marrow is a viscous tissue that resides in the confines of bones and houses the vitally important pluripotent stem cells. Due to its confinement by bones, the marrow has a unique mechanical environment which has been shown to be affected from external factors, such as physiological activity and disuse. The mechanical environment of bone marrow can be defined by determining hydrostatic pressure, fluid flow induced shear stress, and viscosity. The hydrostatic pressure values of bone marrow reported in the literature vary in the range of 10.7–120 mmHg for mammals, which is generally accepted to be around one fourth of the systemic blood pressure. Viscosity values of bone marrow have been reported to be between 37.5 and 400 cP for mammals, which is dependent on the marrow composition and temperature. Marrow's mechanical and compositional properties have been implicated to be changing during common bone diseases, aging or disuse. *In vitro* experiments have demonstrated that the resident mesenchymal stem and progenitor cells in adult marrow are responsive to hydrostatic pressure, fluid shear or to local compositional factors such as medium viscosity. Therefore, the changes in the mechanical and compositional microenvironment of marrow may affect the fate of resident stem cells *in vivo* as well, which in turn may alter the homeostasis of bone. The aim of this review is to highlight the marrow tissue within the context of its mechanical environment during normal physiology and underline perturbations during disease.

Keywords—Mesenchymal stem cells, Marrow progenitor cells, Physiological activity, Osteoporosis, Disuse, Aging, Pressure, Fluid shear, Rheology, Viscosity.

INTRODUCTION

Bone marrow is the soft tissue residing in the cavities of the bones housing the essential pluripotent precursor cells for the living organism. It is the major source of hematopoietic stem cells (HSCs) which have the role of renewing the elements (monocytes, macrophages, neutrophils, eosinophils, erythroblasts,

erythrocytes, megakaryocytes, platelets) in the blood.¹² Adult bone marrow also contains the mesenchymal stem cells which contribute to the regeneration of tissues such as bone, cartilage, muscle, adipose, tendon, ligament and stroma by differentiating into osteoblasts, chondrocytes, myocytes, adipocytes, tenocytes and neuronal cells *in vivo* and *in vitro*.^{6,18,57,76–78,87} Mesenchymal stem cells have been proven to be responsive to mechanical signals such as hydrostatic pressure,^{2,27,86,87} fluid flow induced shear stress⁵¹ and the rheological properties (viscosity) of their environment.^{21,89,93}

Bones are primarily responsible for countering physiological loads. The close proximity of marrow within medullary cavities of bones subject the marrow to physiological loads as well.^{53,79,80,97,116} The key variables of the mechanical environment of marrow due to the external factors can be listed as the intramedullary pressure and the fluid flow generated by pressure gradients. These variables change during regular physical activities.^{23,79,97,123} Intramedullary pressure elevations are reported in response to drugs and steroids.^{3,55,59,90,96} Composition and rheological properties of marrow are also reported to change during aging and drug use.^{39,59} Studies also suggest these properties of marrow to change during aging, osteoporosis or disuse.^{39,90,91} Since these anomalies have the common hallmark of bone loss, the possibility arises that the mechanical environment of marrow may be a key player in homeostasis of bone by way of the mechanically responsive resident stem cells.¹¹⁰

Structure and Function of Bone Marrow

In adults, marrow tissue located in the mid-diaphyseal portions of peripheral bones in the body mostly consists of adipose tissue which imparts a yellowish color (fatty marrow).¹⁰⁴ In the axial skeleton however, the adipose tissue coexist with the hematopoietic tissue in a variable but roughly equal proportion. Bones provide a confined environment for marrow.

Address correspondence to Ozan Akkus, Weldon School of Biomedical Engineering, Purdue University, 206 S. Martin Jischke Drive, West Lafayette, IN 47907-2032, USA. Electronic mail: ozan@purdue.edu

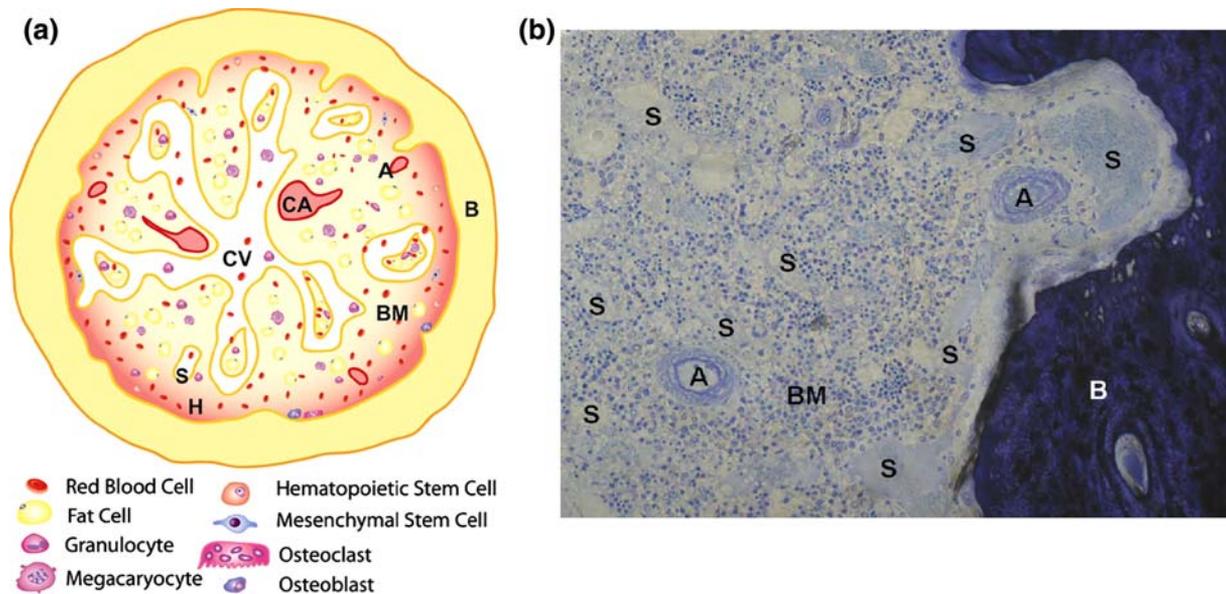


FIGURE 1. (a) Layout of bone marrow in a cross-sectional view of a tubular bone. Bone (B) is surrounding the bone marrow (BM). Central artery (CA) and central vein (CV) are running parallel to each other and longitudinally along the long bone (perpendicular to the plane of the page). The central artery and central vein branch toward the periphery to form arterioles (A) and sinusoids (S) which then combine and join with the central vein. Hematopoietic space (H) is interspersed by the sinusoids. Developing red blood cells and granulocytic cells appear in the hematopoietic space. Megacaryocytes develop subjacent to the endothelium of marrow sinusoids. It is possible to observe the radial distribution of marrow as the yellow marrow in the central regions and the red marrow in the periphery (Adapted from Tavassoli and Yoffey¹⁰⁴ and reprinted with permission of John Wiley & Sons, Inc.). (b) A toluidine-blue stained section taken transversely to the longer axis of a tubular bone. The micrograph displays the endosteal junction between bone and marrow (125 \times). The distribution of abundant number of red blood cells indicates that the bone marrow is hematopoietic (Courtesy of David C. Van Sickle, Purdue University).

Therefore, changes in the volume of active marrow (where hematopoiesis occurs) should be compensated by the expansion of a space-occupying component.³⁰ This component is commonly accepted to be the adipose tissue. When the marrow hematopoietic activity increases, adipose tissue undergoes resorption to provide more space, or vice versa.

Hematopoietic tissue imparts a reddish color to marrow (red marrow) due to the high content of heme chromogen.¹⁰⁴ Red marrow houses the red blood cells, platelets and white blood cells and resides in the flat bones such as skull, ribs, vertebrae, the proximal halves and the endosteal surfaces of the long bones. Red marrow tissue is rich in a variety of cellular components comprised mostly of hematopoietic cells. The hematopoietic cells give rise to monocytes, macrophages, neutrophils, eosinophils, erythroblasts, erythrocytes, megakaryocytes, platelets, and osteoclasts.^{12,15,78,109,117} Marrow's other cellular component is a highly organized stroma that supports the proliferation of the hematopoietic cells.¹¹⁷ The organized stroma is composed of reticular cells which form a spongy framework on which hematopoietic cells are arranged. Upon maturation, hematopoietic cells migrate into the blood stream. Therefore, hematopoietic cells are temporary residents of marrow. Conversely, the stroma remains as a scaffold for the

differentiation and maturation of the hematopoietic cells.¹⁰⁴ Bone marrow also contains mesenchymal stem cells which have been shown to differentiate into osteoblasts, chondrocytes, myocytes, adipocytes and neuronal cells *in vivo* and *in vitro*.^{6,18,57,76-78,87}

The organization of the marrow can be best understood by following its vascular layout. In a tubular bone, the nutrient artery enters the marrow cavity, runs longitudinally in the center, then branches out toward the endosteum of the surrounding bone, leading to specialized vascular structures known as sinuses or sinusoids (Fig. 1).^{7,109} Several of these sinuses may then combine to form collecting sinuses which lead to the central sinus or vein. This vein runs longitudinally next to the nutrient artery. Blood in marrow flows from the center toward the bone and then returns back to the center.^{104,109} This structural configuration yields high numbers of vessels and sinuses in the periphery (resulting in a slower flow rate of blood and higher surface area) where most of the exchange occurs. Therefore, hematopoiesis is maximal in the closer proximities to the bone surface leaving the central parts with relatively little hematopoietic activity (Fig. 1).¹⁰³ Due to this fact, it is possible to observe a transition region between red marrow and fatty marrow radially (red marrow being closer to the endosteal surfaces, Fig. 1).¹⁰⁴ Similarly, a longitudinal macroscopic

distribution is observed as red marrow in the proximal half and fatty marrow in the distal half of the bones.¹⁰⁴ Bone and marrow are connected by vasculature. Bone nutrient vessels enter the marrow cavity to make connections with marrow vessels. Small arteries of marrow also enter the bone, make a loop and return back to where they originated from.^{104,109} A more detailed description of the structural organization and function of marrow can be found elsewhere.^{104,109}

THE MECHANICAL ENVIRONMENT OF BONE MARROW

Hydrostatic Pressure

One of the first studies on the bone medullary pressure reported extensive necrosis of the bone after intramedullary infusion of high pressure saline solution.⁴⁹ Numerous subsequent studies tried to elucidate the possible relationship between the medullary pressure and the hemodynamics of the bone (Table 1). The systemic blood pressure in animals has been reported to be in the range of 110–140 mmHg, whereas the normal intramedullary pressure (IMP) was generally about 30 mmHg which is approximately one fourth of the systemic blood pressure (Table 1). This generalization is known as the one-fourth rule.^{90,91}

It was observed that intramedullary blood flow is directly related to the IMP and was suggested that the IMP is the resultant of the total blood flow entering the bone and the total blood flow leaving it.^{25,90} In addition, marrow pressure depends on the relative degree of resistance to flow between arteries and marrow blood vessels, and between marrow blood vessels and veins. Moreover, IMP and blood-pressure has variations with the phases of respiration such that IMP increases with inspiration and decreases with respiration.⁹⁷ Tondevold *et al.*¹⁰⁸ investigated the changes in IMP in relation to mean arterial pressure. Interestingly, they observed that the medullary pressure remained essentially constant and independent of the mean arterial pressure as long as the latter remained above 81 mmHg. Therefore, it was suggested that there has to be a regulatory system trying to keep the medullary pressure constant, which may be the arterioles of the bone marrow that are supplied by sympathetic nerve fibers with a vasoconstrictor function.²⁴ Shim *et al.*⁹² observed the relationship between IMP and hemodynamics of bone by focusing on the blood supplies of bone. This approach was in contrast to previous studies, which focused on systemic blood circulation. Experiments carried out by Shim *et al.* indicated that IMP rises if the arterial blood supply to the bone increases or venous congestion occurs in the

TABLE 1. Blood pressure and intramedullary pressure (IMP) values reported in the literature.

	Animal	Blood pressure		IMP	
		Location	Value (mmHg)	Location	Value (mmHg)
Stein <i>et al.</i> ^{96,a}	Dog	Femoral artery	110–140	Tibial diaphysis	25–75
Shaw ^{90,a}	Cat	Contralateral femoral or carotid artery	130	Femoral diaphysis	37
Azuma ^{3,a}	Rabbit	Carotid artery	100–110	Femur or tibia	25 (4–70)
Michelsen ^{55,a}	Rabbit	Carotid artery	73–118	Tibia	18–36
Harrelson and Hill ^{32,a}	Mongrel dogs	Femoral artery contralateral Femoral vein contralateral	110–140 2	Mid-diaphysis of femur	35
Shim <i>et al.</i> ^{92,a}	Rabbit	Carotid artery	120	Femur	20–60
	Dog	Carotid artery	130	Femur	40–120
Wilkes and Visscher ^{121,b}	Dog	Femoral artery	134.0 (±13.2)	Tibia	23 (±5.3)
		Nutrient vein	19.3 (±6.3)		
Tondevold <i>et al.</i> ^{108,a}	Mongrel dogs	Left brachial artery	112.9 (±0.9)	Femoral epiphysis	30.7 (±2.6)
				Femoral metaphysis	20.5 (±1.5)
				Femoral diaphysis	25.7 (±1.7)
Thomas <i>et al.</i> ^{107,a}	Rabbit	Not measured	–	Lower femoral diaphysis	33 (7–81)
Bauer and Walker ^{5,a}	Dog	Not measured	–	Femoral diaphysis	27.6 (±15.4)
				Femoral metaphysis	17.6 (±10.5)
				Tibial diaphysis	26.4 (±13.0)
				Tibial metaphysis	17.9 (±11.8)
				Humeral diaphysis	26.2 (±15.8)
				Humeral metaphysis	13.4 (±7.7)
				Radial diaphysis	15.4 (±18.9)
Stevens <i>et al.</i> ^{97,c}	Mouse	Not measured	–	Femur	10.7 (±1.4)

^aIMP was measured with a cannula inserted into the bone in anesthetized animals.

^bIMP was measured with a tonometric pressure transducer in anesthetized dogs.

^cIMP was measured by radiotelemetry in unanesthetized ambulatory mouse.

TABLE 2. The effects of occlusion, epinephrine, norepinephrine, vasodilators, vasoconstrictors and skeletal muscle contraction on intramedullary pressure (IMP) and systemic blood pressure.

	Occlusion		Drugs				Skeletal muscle contraction	
	Arterial	Venous	Epinephrine	Norepinephrine	Vasodilator	Vasoconstrictor	Abdominal	Lower limb
Stein <i>et al.</i> ^{96,a}			↑↓↑	↑↓↑	↓↓	↑↑		
Shaw ^{90,a}	↓↓	↑ →	↓↑	↓↑	↓↓	↑↑		
Azuma ^{3,a}	↓ →	↑ →	↑↓↑↓		↓↓↑ →		↑↓⇒	→
Michelsen ^{55,a,b}	↓↓	↑↑		↓↑	↑↓⇒ ↓↑ →			
Shim <i>et al.</i> ^{92,b}	↓ →	↑ →	↓↓	↓↑			↓ →	↑ →
Stevens <i>et al.</i> ^{97,c}		↑						

↑,↓,⇒: Increase, decrease or no change in IMP; ↑,↓, →: Increase, decrease or no change in systemic blood pressure; ↑↓ or ↓↑: First increase, then decrease or vice versa; vasodilator: acetylcholine, benzyl-imidazoline; vasoconstrictor: amphetamine, histamine.

^aIMP was measured with a cannula inserted into the bone in anesthetized animals.

^bBlood pressure was measured at the femoral artery; vasodilator (acetylcholine) was injected into femoral artery.

^cIMP was measured by radiotelemetry in unanesthetized ambulatory mouse.

All injections were made intravenously; systemic blood pressures were measured at the carotid artery.

limb (Table 2). It was further demonstrated that increased marrow cavity pressure due to venous congestion decreases the blood supply to the bone.⁹² It was also suggested that the elevation of the IMP due to muscle contraction, which mimics physiological activity, may have significance in the maintenance of structural integrity of the bone. Although some researchers claimed to be able to define a relationship between the IMP and the blood circulation in bones, there is neither an agreement nor a consistency in the findings as it can be seen in Table 1. Nevertheless, a common finding in these studies is that the IMP has a pulsatile regime synchronous with the arterial blood pressure and respiration.

There was a significant variation in the intramedullary pressure measurements reported by various authors in the literature (Table 1). This could be due to the differences in the marrow tissue of different species.³ Another explanation to these variations was that the blood channels in the bone marrow were damaged by the insertion of the cannula and it was quite possible that there was a blood pool forming at the tip of the cannula. Therefore, sizes of the damaged channels, the amount of the damage in blood channels and in the extravascular tissue could be causing the variation.³ Similar concerns related to variations in pressure measurements due to lesions formed at the tip of the cannula were raised by various researchers.^{56,92} In order to eliminate the negative effects of hemorrhage or lesions forming, a tonometric pressure transducer was employed which was positioned on the intact endosteal membrane through a cortical hole of about 7.3 mm in diameter.¹²¹ Although the enhancing characteristics of the measurement technique were not discussed in detail, less variation was observed in the IMP measurements (Table 1). However, the difficulties in the surgical techniques used in this study caused

puncture of the endosteal membrane and gross trauma in half of the animals. Due to the advances in sensing electronics and telemetry, it has been recently possible to conduct *in vivo* IMP pressure measurements on unanesthetized ambulatory mice by implantation of a radiotelemetry pressure transducer.^{97,98} The transducer was composed of a 0.4 mm diameter catheter, which was inserted through a 0.5 mm hole in the femoral cortex and sealed with tissue cement. The catheter was connected to a transmitter, which was secured in the peritoneal cavity, onto the abdominal wall. The new technique was successfully employed for long term, *in vivo* measurements of IMP in venous ligation and hindlimb suspension mice models (Tables 1 and 2). This new technique has proved to be more efficient in yielding *in vivo* pressure measurements with more accurate readings.

The response of the mechanical environment of the bone medullary cavity to various factors has revealed that the marrow environment is quite susceptible to external factors. In addition, this response has been shown to be quite robust in its recovery towards its normal state after its dynamics were disturbed. The studies in this regard aimed to elucidate the hemodynamic changes in bone marrow due to external effects such as occlusion of regional vessels, injection of epinephrine, norepinephrine, acetylcholine, pressor and depressor drugs, and skeletal muscle contraction (Table 2).

It is commonly anticipated that physiological loading (i.e. walking or running) perturbs the IMP.^{53,79,80,97,116} This supposition was tested by externally stimulating skeletal muscles of anesthetized animals to mimic the physiological loading conditions. The results (Table 2) showed that IMP rises due to muscle contraction. In a more recent *in vivo* study, the effect of hindlimb suspension and venous ligation on

the medullary pressure was successfully measured by telemetry in ambulatory mice.⁹⁷ In this study, surgical venous ligation increased the IMP by 25%. In addition, IMP decreased by 23% in normal mice and decreased by 33% in ligated mice upon hindlimb suspension (Table 2). In general, arterial occlusion yielded a decrease while venous occlusion yielded a rise in the medullary pressure, both of which verify the direct relationship between the blood flow into the bone and the IMP (Table 2). The effects of vasodilator, vasoconstrictor drugs and steroids on marrow pressure were investigated on anesthetized animals.^{3,55,59,90,96} Vasodilator drugs tend to lower both the marrow and the systemic blood pressure while vasoconstrictor drugs tend to increase both of them (Table 2). Miyanishi *et al.*⁵⁹ attempted to relate steroid-induced osteonecrosis with intraosseous pressure rise in rabbits. They observed that steroid treatment significantly increased marrow pressure, decreased bone blood flow and also caused fat cell enlargement.

Rheology

It has recently been indicated that marrow viscosity is a critical parameter modulating the shear stresses experienced by the trabecular surfaces in the vertebral bodies due to vibratory loads.¹⁹ Since the potential rheological changes in the marrow due to bone diseases and aging is likely to affect the shear stresses experienced by the progenitor cells in marrow, it is essential to quantify and characterize these parameters. Bone marrow (bovine) has been shown to present Newtonian fluid characteristics (i.e. constant viscosity which is independent of shear rate) at near body temperatures.¹⁰ It should be noted that marrow has a slightly lower temperature (1.6–4.8 °C below) than normal body temperature in humans.⁷³ Bryant *et al.*¹⁰ observed the dependence of bovine bone marrow's rheological properties on temperature and anatomical

location. The measured viscosity of the proximal marrow at 35 °C (≈ 400 cP, viscosity of water is 1 cP) was found to be about ten times that of the distal samples (≈ 40 cP) which can be associated with compositional variations of marrow along the bones (Table 3).²⁰ Noting that the proximal ends of bones contain red marrow, whereas the distal ends contain yellow (fatty) marrow¹⁰⁴; it is reasonable to infer that the increased fat content in marrow may reduce its viscosity. Interestingly, removal of cell debris, blood cells and other granular matter from the marrow by centrifugation decreased the dependence of its viscosity on temperature.¹⁰ Considering the durations of viscosity measurement intervals of as long as 6 h; the potential effect of coagulation of marrow samples was not discussed in this study.¹⁰ In a more recent study on the measurement of femoral bovine marrow viscosity with an implantable wireless method, a viscosity value of 123 cP was reported.³¹ The only study on the viscosity of human bone marrow (calcaneal marrow which was reported to be mainly yellow or fatty) reports values in the range of 37.5 cP (at 36 °C) and Newtonian fluid characteristics (i.e. viscosity is not dependent on the shear rate).¹⁷ However the authors report non-Newtonian behavior for the bone marrow specimens with red components (i.e. higher blood cell concentration).¹⁷ This finding is not surprising as it is known that human blood displays non-Newtonian characteristics.²⁶ In addition, the density of red bone marrow (1.06 g/cm³)¹²⁰ has been reported to be comparable to that of blood (1.05 g/cm³).²⁶ It should be noted that there is limited information available in the literature on the density of fatty marrow (0.89 g/cm³)³¹ which is comparable to that of fat tissue (0.92 g/cm³).¹²⁰ The viscosity values reported in the literature for bone marrow and blood are summarized in Table 3.

The variation of marrow composition along the bones and in the different parts of the body is still a

TABLE 3. Viscosity measurement values reported in the literature for bone marrow and blood.

	Subject	Location	Temperature (°C)	Value (cP)
Bone marrow				
Bryant <i>et al.</i> ¹⁰	Bovine	Proximal radius	35	400 ^a
	Bovine	Distal radius	35	44 ^a
	Gurkan and Akkus ³¹	Bovine	Femur	123 ^b
	Davis and Praveen ¹⁷	Human	Calcaneus	37.5 ^c
Blood				
Eguchi and Karino ²⁶	Human	–	37	66 ^d

^aMeasurement was obtained with cone and plate viscometer.

^bMeasurement was obtained with parallel plate rheometer.

^cMeasurement was obtained with controlled oscillatory flow within a straight, cylindrical tube with circular cross section.

^dMeasurement was obtained with falling ball viscometer at a shear rate of 0.189 s⁻¹ on a blood sample with a hematocrit value of 41%, and a density of 1.05 g/cm³.

question to be answered. This variation can be explained by the temperature dependence of marrow. Marrow composition is highly affected from variations in temperature. Huggins *et al.*³³⁻³⁵ evidenced that the fat content of bone marrow in the limb bones (femur, radius) are higher than the bones in the central parts of the body (ribs, vertebra). This was suggested to be associated with greater body temperatures in central bones. Similarly, Weiss *et al.* demonstrated that in summer times, the bony exoskeleton of the nine-banded armadillo (*Dasybus novemcinctus*) displayed a red or erythropoietic marrow; whereas in winter times, when the ambient temperature is low, the marrow was yellow or fatty.¹¹⁸

The rheological properties of bone marrow in different parts of the body and in osteoporotic, disused or aged bones are not known. *In vivo* monitoring of marrow in bone disease and disuse models can provide more realistic values due to the elimination of effects of extraction. The outcomes of future investigations in this area may prove to be valuable for researchers trying to simulate the natural environment of stem and progenitor cells in mechanically stimulated cell cultures in an effort to better understand bone loss.

While the intramedullary pressure and the rheology of marrow are relatively well investigated, the shear stress in the marrow during ambulation is largely unknown. Shear stress within the marrow as well as between the marrow and the endosteal bone may play a role in terms of modulating the biological response of marrow resident cells and the endosteal lining. The deformation and flow of marrow due to mechanical loading and associated effects on the stromal cells is an area open to investigation and it needs to be studied further.^{51,100}

EXTERNAL INFLUENCES TO THE MECHANICAL ENVIRONMENT OF BONE MARROW

Effects of Physiological Activity and Loading

When the mechanical loading on bones is removed due to extended periods of inactivity, such as bed rest or during space-flights, bone mineral density decreases.^{50,84,122} On the other hand, elevated amounts of loading on bones due to exercise causes increased bone mass and bone turnover.^{16,45,61,62,65,112} Even though there is *in vivo* evidence that exercise induced mechanical loading enhances bone mass, the cellular and molecular mechanisms underlying this fact are still being studied. Bone homeostasis is a balanced system of formation by osteoblasts and resorption by osteoclasts. Osteoblasts are the bone forming cells that originate from mesenchymal stem cells residing in

marrow. Whereas osteoclasts are the bone resorbing cells that originate from the hematopoietic stem cells in marrow.¹³ Bone marrow mechanical environment is known to be affected from loading of bones with variations in intramedullary pressure, intramedullary and interstitial fluid flow.^{53,74,80,97,116} Since marrow is housing the precursors of osteoblasts and osteoclasts, the changes in the mechanical environment of marrow due to physiological activity and loading may play a role in bone homeostasis.

The earlier studies on the effects of loading on the mechanical environment of bone marrow tried to elucidate the strengthening characteristics of marrow as a slightly compressed liquid in the trabecular regions. It has been suggested that intertrabecular fluid (marrow) pressurized by compressive deformation of the bone may provide load bearing capabilities.^{9,40,41} The related *ex vivo* and *in vivo* studies proved that marrow inside the trabecular regions of femoral head provides stiffness to subchondral trabecular bone.^{70,71} It was also suggested that bone marrow acts as an intermediate transferring the external loads by means of pressurization to trabecular bones, which are acting as trusses in subchondral regions. Therefore, the trabeculum bears only the modest pressure difference across it as a tensile load.⁹⁵

Researchers have used both *in vivo* and *in vitro* models to study the effects of physiological loading and activity on bone marrow. Kumar *et al.*⁴⁶ loaded the fixed femurs of rats *in vivo* applying loads ranging from 0 to 12.25 kg for 1 min. They observed pressure values to rise about two-fold (12–14 mmHg increase) upon loading. They also studied contraction of the quadriceps muscles with electrical stimulation, resulting in a pressure increase of 60 mmHg.⁴⁶ Another study loaded the tibiae of the sheep in impact *in vitro* with loads of 2000 N for durations of 0.015 s and observed elevations up to 300 mmHg.⁷ Downey *et al.*²³ studied the effects of *in vitro* compressive loading in the human femoral head. The extracted human hips from cadavers were subjected to load by means of a mechanical testing device. They observed the IMP rise by 55 (\pm 66) mmHg per 980 N of load applied over 0.1 s. The load values and the application durations were estimated to be comparable to physiologic conditions. They also presented an interesting finding about an osteoporotic bone with a much higher pressure rise of about 220 mmHg as a result of a relatively less load of 590 N. However, due to the lack of blood flow in the bone resting IMP of the bones was zero and the resemblance of these loading conditions and models to natural physiological loading conditions are disputable. These studies, regardless they are *in vivo* or *in vitro*, apply artificial external loads with assumed similarity to physiological loads. Moreover, *in vitro*

studies lack blood circulation in the bone and in the muscle tissue, both of which may have potent effects on the pressure of bone marrow during physiological loading. An ideal study investigating the effects of physiological loading on the mechanical environment of bone marrow should employ slightly invasive or noninvasive instrumentation which at the same time facilitates continuous measurements in unanesthetized ambulatory animal models. Furthermore it should be noted that the magnitudes of the loading induced IMP is dependent on factors such as loading rate, viscosity of marrow and porosity of the surrounding bone.^{19,80} Therefore these factors should be taken into consideration when evaluating the relative magnitudes of loading induced IMP.

An explanation for bone loss due to lack of exercise and physical activity is decreased medullary cavity pressurization.⁸⁴ It has been reported that bone loss is not equal throughout the skeleton in long-term bed rest. Bone loss is the greatest in lower extremities, where the medullary pressure is significantly decreased due to lack of activity. On the other hand, bone formation is observed in the head, where the intracranial pressure is increased due to body orientation and shift of body fluids.⁵⁰ This suggestion has recently been supported by a mouse hind limb suspension model with *in vivo* femoral IMP measurements for extended durations.⁹⁷ The study aimed to infer the interstitial fluid flow changes due to pressure gradients between the endosteum and periosteum. A decrease of 23% in the IMP was observed upon hindlimb suspension. Although the correlation between the IMP and the bone formation or bone loss was not reported, it was shown that medullary pressure is sensitive to disuse with an *in vivo*, unanesthetized and ambulatory animal model. Correspondingly, a strengthening treatment technique for osteoporotic long bones has recently been put forward, which elicits cortical bone formation in the femoral neck region of mice by means of knee loading *in situ*.^{124,125} It has been verified by a following study that the intramedullary pressure is altered in synchrony with the knee loading.¹²³ It should be noted that 2/3 of the blood supply of cortical bone is provided by the endosteal surface.^{80,102} It has also been shown that the variations and oscillations in IMP play an important role on the loading induced fluid flow developed in the interstices of the cortical bones as well as of the trabecular bones.^{97,101,124} Therefore it is likely that the variations in the IMP not only affect the resident pluripotent stem cells but also serve as one of the players which are driving the fluid flow within the interstices of cortical bone by way of generating pressure gradients between the envelopes of bone. This flow is essential to provide nutrients and to remove the metabolic byproducts of osteocytes resident in mineralized cortex.

Effects of Osteoporosis and Aging

Osteoporosis is a disease of bone in which fracture susceptibility is compromised by decreased bone mineral density. It mostly involves the hip, the wrist and the lumbar vertebrae.⁸¹ It is estimated that over 20% of women and 7% of men over the age of 50 have osteoporosis. Osteoporosis is responsible for over 1.5 million fractures in the United States annually.⁷² It has been evidenced that there is a significant change in marrow composition and mechanics due to osteoporosis and aging.^{39,90,91,115} The only study in this regard stated that the IMP in the tibial diaphysis of children with paralytic osteoporosis approximates the arterial blood pressure. Whereas the tibial IMP of the healthy children with the same age was only about one quarter of the arterial blood pressure.^{90,91} In addition, the adipose tissue fraction in marrow increases significantly with osteoporosis,^{39,115} which may be yielding to obstruction of blood circulation.⁵⁹ Increased adipose fraction, thus decreased viscosity may be causing the overall shear stress in the medullary cavity to decrease in osteoporosis. Potential elevations in pressure or decrease in shear stress in osteoporotic bones may contribute to osteoporosis by way of altering the milieu of the bone marrow progenitor, precursor and stem cells.

Osteoblasts and adipocyte cells both originate from the mesenchymal stem cells.⁷⁶ It has been suggested that the commitment of stem cell fate is integral with mechanical cues experienced in developmental and adult contexts, embodied in cell shape, cytoskeletal tension and RhoA signaling.⁵⁴ RhoA is a small GTPase protein known to regulate the actin cytoskeleton in the formation of stress fibers. It has been shown that human mesenchymal stem cells subjected to different mechanical environments differentiate into either osteoblasts or adipocytes depending on the RhoA activity (RhoA active: osteogenesis, RhoA negative: adipogenesis).⁵⁴ Likewise, adipocyte tissue volume in bone marrow has been shown to increase with aging and in patients with osteoporosis.^{39,115} Therefore, it can be hypothesized that osteoporosis may be due to a greater portion of mesenchymal stem cells differentiating into adipocytes than osteoblasts due to the changes in the cell properties and/or the mechanical environment of the marrow.

EFFECTS OF THE MECHANICAL ENVIRONMENT ON STEM AND PROGENITOR CELLS

It should be noted that the responses of the marrow derived progenitor cells to physical factors such as hydrostatic pressure, fluid shear and the rheology of

the environment have been extensively studied *in vitro*. The question about the natural physiological and altered mechanical conditions of these cells in the bone marrow remains to be answered. Therefore, the review focuses on the effects of mechanical loading to stem and progenitor cells *in vitro* to illustrate that stem cells are responsive to mechanical cues. However, it remains to be determined as to whether this mechanical responsiveness exists *in vivo*.

It is commonly accepted that stem cells' microenvironment, which includes biochemical and biomechanical factors, has an important role on their differentiation and phenotypic expression. Even though most studies in the literature place an emphasis on growth factors and cytokines, it has been evidenced that the differentiation of precursor, progenitor and stem cells are also significantly influenced by mechanical factors.²⁷ The main mechanical signals that are accepted to be effective on marrow precursor cell proliferation and function are: hydrostatic pressure and fluid-flow induced shear.^{27,93} The viscosity of marrow is also important since it directly relates to the fluid shear stress magnitudes experienced by the cells. The influences of these factors are considered to be significant in regulating the stem cell phenotype and may have synergistic and/or supplemental effects in combination with the biochemical factors. The employment of such mechanical effects in specially designed "bioreactors" may enhance current standard biochemical signaling pathways for promoting engineered tissue growth.^{27,42,88}

Marrow-derived mesenchymal stem and/or progenitor cells offer novel treatment techniques in tissue engineering research. They are already cultured *ex vivo* in mechanically active environments for various purposes, such as tissue engineering of bone and cartilage.^{6,15,47,69,77,93,94,99,114} The main goal of moving from conventional 2-D methods to 3-D mechanically active systems is to attain more physiological (or natural) culture environments for the cells. Although these studies prove improvements over conventional culturing techniques, they are far from *in vivo* conditions in efficiency.

Hydrostatic Pressure

There are two main reasons why hydrostatic pressure is considered as an important stimulating factor for bone marrow progenitor and stem cells. First of all, marrow derived mesenchymal progenitor cells can express chondrogenic phenotypes under appropriate hydrostatic pressure conditions. There are numerous *in vitro* studies subjecting marrow-derived mesenchymal stem cells to high hydrostatic pressures (750–75,000 mmHg) in an effort to induce chondrogenic

phenotype.^{1,2,57,58,68,87} Even though the chondrogenic differentiation of the progenitor cells does not take place in marrow cavity, those studies are related in indicating the sensitivity of the progenitors to the magnitude and the mode (cyclic, intermittent or static) of loading. In general, higher pressure (~75,000 mmHg) over lower (~750 mmHg) and intermittent loading over static have proved to be more effective in chondrogenesis.^{57,58} Second, hydrostatic pressures in the medullary cavities of long bones due to physiological loading (50–200 mmHg rise)^{8,23,46,84,110} have osteogenic effects.⁸⁶ It has been shown that constant hydrostatic pressure applied *in-vitro* at physiologic levels (30–60 mmHg) decreases osteoclast formation.⁸⁵ Similarly, cyclic pressures of 75–300 mmHg in magnitude decreases formation of osteoclasts from their progenitors and decreases bone resorptive activity by osteoclasts *in vitro*.⁶³ Furthermore, this loading scheme enhances osteoblast functions pertinent to new bone formation by stimulating both synthesis and deposition of collagen accompanied by increased accumulation of calcium-containing crystals.^{63,64} A general conclusion that can be drawn from those *in vitro* studies is that the osteogenic response of the cells are not always linearly proportional to the value of the mechanical signals, rather there is an optimal amplitude interval for each loading pattern and frequency. Limited number of *in-vivo* studies in this regard report similar results. Oscillatory hydrostatic pressure (60 mmHg, 20 Hz) applied *in-vivo* for 10 min per day for 4 weeks to avian ulnae elicited new bone formation on the periosteal surface⁷⁹ possibly due to increased intracortical fluid flow as suggested by the authors. It was also suggested that since the applied loading regime has bone forming effects; it should be similar to the physiological loading conditions. Similarly, intramedullary hypertension due to venous occlusion (pressure increased to about 28.7 mmHg from 15.5 mmHg) has periosteal (138%), endocortical (369%) and cancellous (889%) bone forming effects at the caprine tibial metaphysis.¹¹⁹ One of the suggestions on how the marrow pressure affects bone formation is that the progenitors in the marrow may be directly reacting to marrow pressure or extracellular fluid flow (developed by pressure gradients) by differentiating into osteoblasts and forming new bone.^{51,110} On the other hand, it is suggested that nitric oxide is imperative in signaling of mechanically induced bone formation.^{28,75,113} Nitric oxide is a signaling molecule and it is synthesized by nitric oxide synthase which is produced by osteoprogenitor cells in bone marrow, by osteocytes in bone and bone lining cells.²⁹ Therefore, it is probable that the production of this signaling molecule by the osteoprogenitor cells in the marrow is mediated by bone marrow pressure or pressure gradients.¹¹⁰

Fluid Shear

Mechanical loading and bending of bones cause strain gradients as well as local pressure gradients in the bone and in the medullary cavity that can drive the interstitial fluid flow^{53,74,80,116} and can result in shear stresses on the endosteal surface. Fluid flow induced fluid shear on bone marrow derived cells has been investigated extensively to assess its effects on cell differentiation, proliferation and function.^{6,15,51,63,94} Besides, it has been evidenced by a number of researchers that interstitial fluid flow may play an important role in bone remodeling, formation and adaptation.^{11,37,53,67,79,111} The mechanisms of flow-induced remodeling have been studied *in vitro* by subjecting osteoblasts,^{22,37,43,48} osteocytes^{37,44} and osteoclasts^{53,85} to fluid shear. The studies on osteoblasts and osteocytes showed that several osseointegrating agents are stimulated by fluid flow such as nitric oxide,^{38,75} prostaglandins E2 and I2,^{43,52,66,67,82} cyclic adenosine monophosphate,⁸² intracellular free calcium,^{14,22,36,37,64,86} inositol triphosphate,^{36,82,83} and transforming growth factor β .^{27,43,57,58} On the other hand, osteoblast-mediated mineralization is preceded by osteoclast-mediated resorption with osteoclast resorption rates being about 20 times higher than osteoblast deposition rates.¹⁰⁶ Therefore, osteoclast function may dominate the dynamic osteoclast-osteoblast balance that regulates bone turnover and degradation. Moreover, it is suggested that physiological load induced fluid flow in cortical bone is radially outward from endosteal surface to the periosteal surface,^{60,80} which makes it difficult for osteoblast or osteocyte secreted mediators to diffuse against the current and reach the osteoclasts or osteoclast progenitors in the marrow cavity.⁵³ The osteoprogenitor cells are reported to be residing in bone marrow, one to three cell layers away from the endosteal surface.¹⁰⁵ Accordingly, it has been suggested that osteoclasts and their precursors, which are located in the close vicinity of endosteal surface, may be fluid flow induced shear sensitive and that osteoclast remodeling activities may be under the control of autocrine factors.⁵³ Bone marrow derived osteoclast-like cells are mechanosensitive to fluid flow induced shear and secrete autocrine factors, such as nitric oxide, prostaglandins E2 and I2, which can regulate local resorptive activity.⁵³ Therefore, fluid flow induced shear developing on the endosteal surface in the medullary cavity may be the significant stimulant for osteoprogenitors in the marrow that recruits them to bone formation sites. Human marrow stromal cells subjected to oscillatory fluid flow of 1 Hz are shown to have increased proliferation rates,⁵¹ which means more osteoprogenitor cells to participate bone formation process. Similarly, the sensitivity of the cells in fluidic

environment to flow frequency has been studied to find the potent optimal frequency values for cell phenotype determination.⁴ However, the flow mode that is naturally experienced by osteoprogenitors in marrow has not been revealed yet.

Rheology

Viscosity of the environment in which cells reside is important since it directly affects the shear stresses experienced by cells if the medium is flowing. The studies in this field investigate the viscosity of the environment in mechanically active cell cultures or perfusion bioreactors.^{21,89,93} In one such study, the effect of medium viscosity on marrow stromal osteoblastic cells seeded on 3D fiber meshes was studied by adding dextran (a complex, branched polysaccharide) to flowing medium in a perfusion bioreactor.⁹³ Increasing medium viscosity with constant flow rates resulted in 2- to 3-fold increases in the shear stresses experienced by the cultured cells without changing chemotransport characteristics significantly. It was reported that increased medium viscosity not only enhances mineralized matrix deposition but also provided a better matrix distribution in the porosity of the 3D scaffolds. In a similar study, the effect of fluid flow induced shear and chemotransport on bone cells was studied in oscillatory flow.²¹ The medium viscosity was varied to obtain different shear stress values on the cultured cells under constant flow rates. It was shown that fluid flow induced chemotransport and shear stress acting on the cells play a synergistic role to elicit cell response to oscillatory fluid flow induced shear stress. In an effort to control the cell aggregate sizes in neural stem cell cultures, researchers tried to alter the kinematic viscosity of culture medium by adding dextran and carboxymethylcellulose.⁸⁹ The results indicate that viscosity is an important parameter to consider for scale-up of stem cell bioreactors. The viscosity values of the media employed in these studies were in the range of 1–4 cP, whereas the reported viscosity values for bovine marrow was in the range of 44–400 cP.¹⁰ Therefore there is a need to revisit these studies and assess stem cell response at these higher viscosity media.

CONCLUSION

Bone marrow mechanical environment is susceptible to external effects such as physiological activity and disuse. Moreover, there is a potential relation between the bone diseases such as osteoporosis, and aging or disuse-related bone loss and the marrow composition and mechanics. The changes in the bone marrow mechanical environment is likely to be effective on the

occupant precursor and progenitor cells, which are accepted to be responsive to mechanical factors such as hydrostatic pressure, fluid shear and the viscosity of their environment. Although the effects of these mechanical factors and viscosity on mesenchymal stem and progenitor cells are being widely investigated *in vitro*, the naturally occurring and altered cues in the cells' natural environment (bone marrow) have not been well characterized yet.

Bone marrow mechanical environment can be completely defined by quantifying and characterizing the hydrostatic pressure, fluid flow induced shear and viscosity in natural and altered conditions. The results of these studies can be compiled to generate physiologically relevant *in vitro* mechanical environments for cell cultures, stem cell bioreactors and computational models to better understand the effects of mechanical signals on stem, progenitor and precursor cells; and potentially the nature of associated bone diseases.

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