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Biomimetics in Bone Cell Mechanotransduction: Understanding Bone's Response to Mechanical Loading

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1. Introduction

In biomimetics work, bone has often been cited as inspiration, ranging from the architectural influences in the Eiffel Tower to aerospace influences and adaptive strengthening of wing structures subjected to overload. However, the intricate complexity of bone itself means that there is still much to learn regarding how this composite can adapt so well to its loading environment. Moreover, many believe identifying and understanding these pathways and mechanisms holds the key to eradicating metabolic bone diseases such as osteoporosis and osteopetrosis. Specifically, as bone researchers, our goal is to understand how the bone cells which are responsible for the formation/destruction of bone coordinate their activities. In the laboratory, isolated cells (*in vitro*) and animal (*in vivo*) models are employed based upon unique advantages. *In vitro* systems have the advantage of isolating key factors to be studied but given their simplicity, their relevance to the *in vivo* world is questionable. *In vivo* systems have the advantage of clinical relevance and long-term study, but given their complexity, tweezing out the effects of the isolated loading events is difficult. Recently we have proposed using organ culture (*ex vivo*) bone models to study the effects of mechanical loading on bone cells. In these systems, whole bones are maintained in culture and the effects of an isolated load may be studied. The goal in essence is to study the bone cell response in a system that mimics the biological event. The intent is to increase the relevance of *in vitro* studies by maintaining and studying the response of the cells as they interact with each other (and other cell types) in a native, three-dimensional matrix with intact communication networks, a biomimetic system. However, these models also have disadvantages. Specifically, cut off from any blood supply, they are a dying organ and the question remains to be answered if they remain viable for a long enough period of time to be useful models for dissecting the response of bone cells to mechanical loading. This chapter will introduce the reader to the research area of bone mechanotransduction with a focus on engineering mechanics and address the validity of *ex vivo* systems as biomimetic models in comparison to *in vitro* and *in vivo* approaches. Finally we will look at the application of the organ culture approach and assess its usefulness in modeling the clinical procedure known as distraction osteogenesis.

When one thinks of bone and mechanics, the common images conjured up are of biomechanics tests. In bone biomechanics, mechanical testing principles and techniques are applied to, for instance, testing a bone under bending or torsion to determine material and structural properties, or the bone may be used as a holder to determine useful life of an implant or fracture fixation system. In bone biomechanics tests the bone is viewed largely as a static structure and the mechanical loading is used to determine an endpoint condition or value (strength, toughness, shear modulus, fatigue life). In contrast to biomechanics, in the field of bone mechanobiology it is recognized that bone is a highly dynamic structure which is subjected to mechanical forces/loading and that these loads are necessary, even critical, for bone growth, maintenance and function. Furthermore, bone metabolic diseases, such as osteoporosis, may be associated with the inability of the bone cells in the aging skeleton to sense and/or respond to mechanical loading levels that are sufficient to maintain bone quality in the younger skeleton. In bone mechanobiology, it is recognized that bone is a highly dynamic composite and mechanical loading is an input or impetus critical for normal bone quality and quantity and its maintenance. More specifically, since it is not the bone that responds to the mechanical loading but the bone cells that are responsible for the bone formation/destruction that respond to mechanical loading, mechanotransduction is a focused area of study in the bone mechanobiology field aimed at identifying and understanding the mechanisms and pathways by which bone cells sense and respond to mechanical stimulation in normal and abnormal (disease, implant introduction, spaceflight) loading environments.

Mechanotransduction work relies heavily on 'testing systems' that can provide load/stimulation to a cell, tissue, organ or animal model system. These testing systems provide an accurate and reproducible load/stimulation to the model and vary greatly in cost, function and flexibility. Given the engineering intent of this article, the testing platform will be explained in some depth. Commercial testing machines are uniaxial and biaxial with the latter comprising both torsional and rotational capabilities. While these systems can be quite expensive, they offer a degree of accuracy and precision under a variety of loading controls (displacement, load and strain) that is unparalleled. However, given the nature of extramurally funded research and the inclusion of engineers in the mechanobiology field, much of the mechanical testing system development is done in-house and a variety of single-purpose systems have evolved for these studies. While describing all these systems is not possible, and several excellent articles utilizing these systems are available to the interested reader (Rubin and Lanyon, 1984; Rubin and Lanyon, 1985; Turner, et al., 1991; Hillam and Skerry, 1995; Brown, 2000; Gross, et al., 2002), we will describe in some detail the development of a platform used in our lab and then utilize this system to explain the applications to the mechanotransduction work and the accompanying engineering strengths and limitations of the systems. It is important, given the multidisciplinary nature of the field that engineers involved in this research appreciate the limitations of the loading devices and communicate this to the biological scientists. The converse is also true, engineers need to be made aware of the biological limitations of the systems to better design and develop systems that accurately mimic the physiologic environment.

The initial platform designed in-house accommodated standard biomechanics tests including: bend testing of bones, compression testing of hard tissues, tension testing of soft tissues, and mini-implant evaluation and mechanotransduction tests, including: *in vivo* exercise loading and fluid shear and substrate deformation of bone cells. This device is a uniaxial system; torsional loading is made possible with the addition of a rack and pinion

fixture. While most commercial systems are single axis (uniaxial) machines built on a fixed base, we opted for a movable base and found that an inexpensive way to create the two main components of the platform, the linear, vertical motion and the base frame was to purchase a slide and milling machine table. The milling machine table, given its routine use in machine shops offered a very cost-effective alternative to expensive stereotaxic platforms without compromising accuracy or precision. The slide and table are connected via aluminum plates. All connections are slotted with keyways to make assembly reproducible. To reinforce the machine for larger loads, side plates running the vertical length of the slide may be added (not shown in Figure 1). A range of transducers accommodate a variety of testing needs. Load cells range from 50 gm to 445 N; torque cell capacity is 176.5 Nmm (25 oz-in); and, displacement sensors accommodate 5 and 25 mm of travel (Saunders and Donahue, 2004). As is typical of biomechanical testing systems, machine deformation is largely unaccounted for, but assumed to be negligible given that machine stiffness is much greater than specimen stiffness (Currey, 2009).

While the system is highly flexible and cost-effective, it is extremely important to acknowledge the limitations of the in-house device. For instance, the device does not have feedback and as built can only be run under displacement control. While this does not negate the usefulness of this system for relatively rigid fracture (single load to failure) testing, it does affect highly elastic and viscoelastic materials and the fatigue (multiple loading cycles to failure at loading levels below that inducing fracture) of these materials. In the case of displacement control, the particular slide chosen is controlled by a servo motor that operates under a series of user-developed programs that control for variables such as displacement, velocity and acceleration. Again, while this does not greatly affect a bone fracture test, the device is not a convenient tool for applying a frequency driven waveform, such as a pure sinusoid. For these needs, user-defined programs are curve fit to characterize oscillatory waveforms that approximate within reason (< 5%) a desired sine wave. It also requires the adaptation of ASTM standard protocols requiring load control to a displacement control model. In the case of feedback, the simplest way of envisioning this concept is that feedback provides the machine with the information to understand or 'eyes' to 'see' the material/specimen that is being tested. For instance, feedback settings (such as rates, gains and loops) enable a machine (running under load control) to quickly adjust to changes in the material/test to maintain a constant load. It is not hard to appreciate how different this adjustment would be for the same constant load test on a steel bar in comparison to a rubber strip. And the need for this information becomes critical when testing highly elastic/viscoelastic (high degree of hysteresis) materials under fatigue to ensure that the load is efficiently reached and maintained. In the absence of such feedback, the overshooting/undershooting of the load can lead to very erroneous data. While this does not negate the utility of a system without feedback, it does put the responsibility with the operator to understand the limitations of the testing system and determine if reliable data can be obtained with a particular platform.

Once the basic platform is developed, as with biomechanics, mechanobiology research reduces to developing fixtures/models that accurately address the question at hand. In biomechanics this may be as simple as developing a compression platen that correctly distributes an even load across the surface of a scaffold (Figure 2), or a four-point bend fixture that concomitantly applies load to all four points of contact on a long bone, regardless of geometric symmetry. In mechanotransduction, this process is generally more

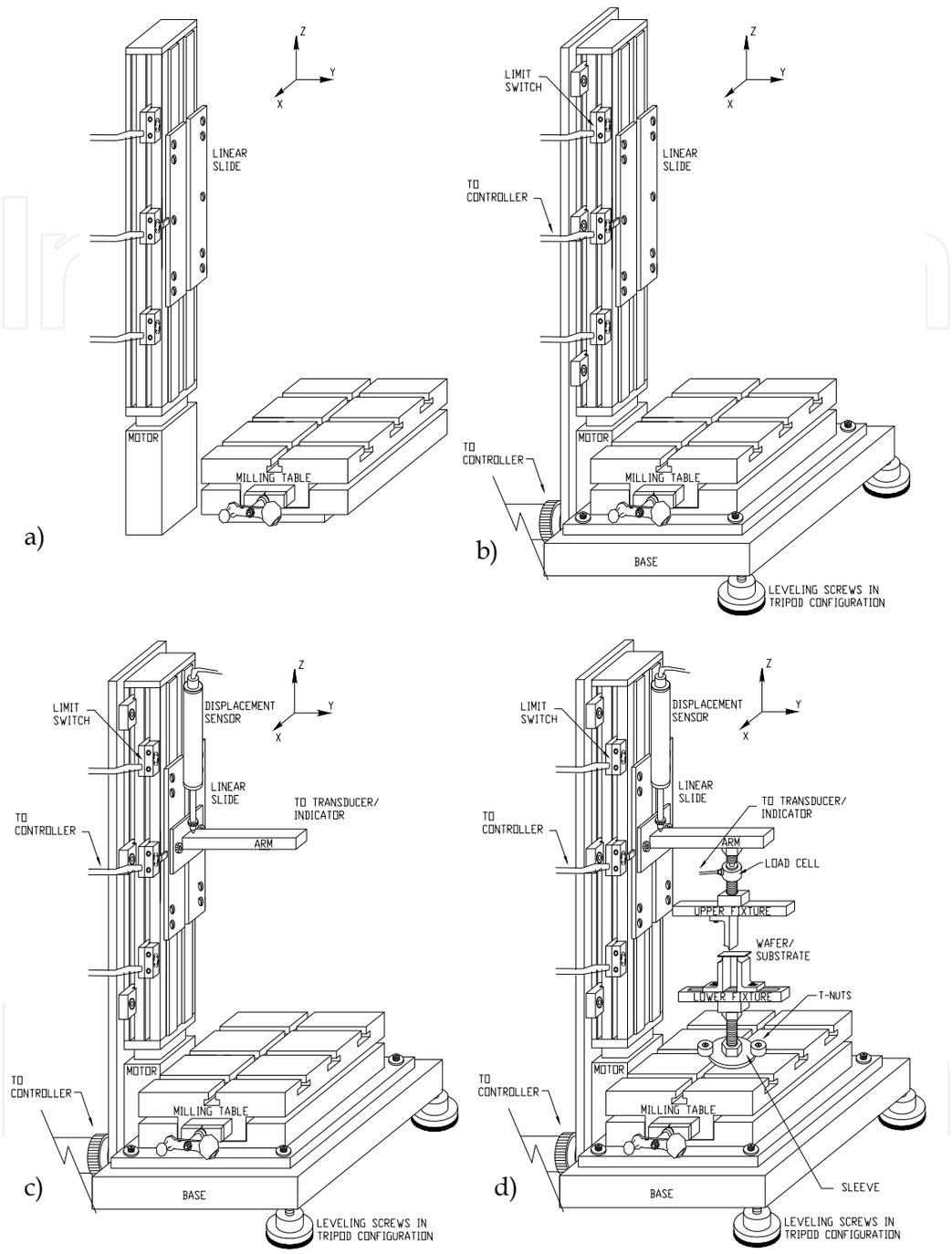


Fig. 1. (a) Small-scale loading machine designed around a commercially-available linear slide and milling machine table. (b) Aluminum plates were fabricated to connect the slide and table with keyways for easy and reproducible assembly. (c) An arm attached to the slide and t-slots in the milling machine table enables a variety of fixtures to be assembled in the platform, such as those shown for three-point bending (d).

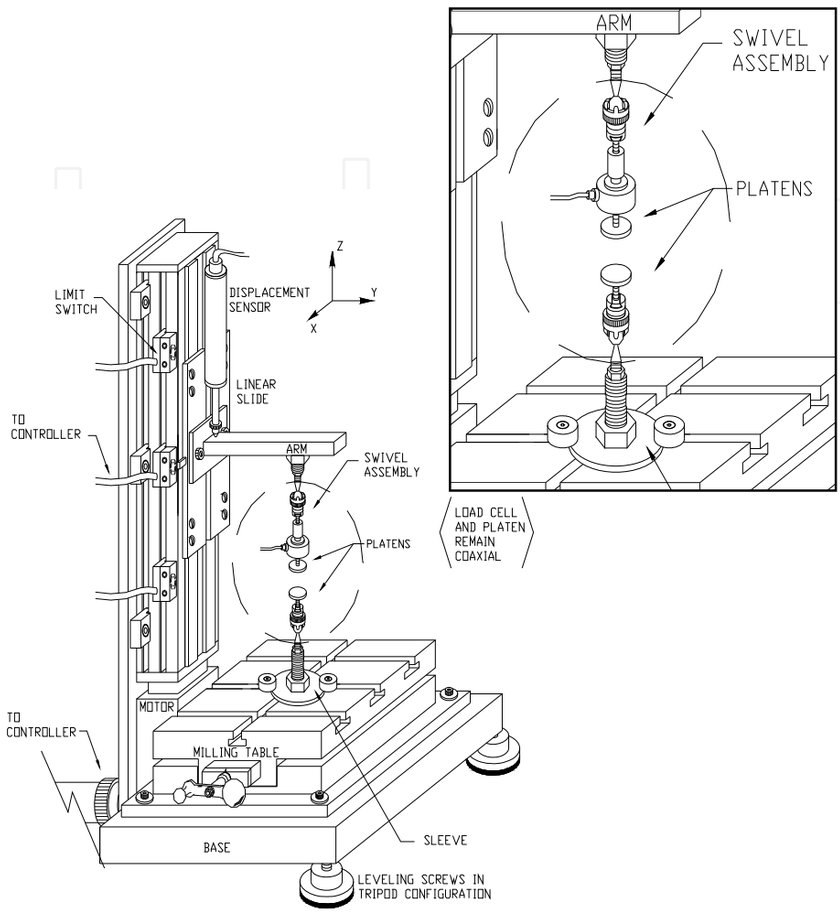
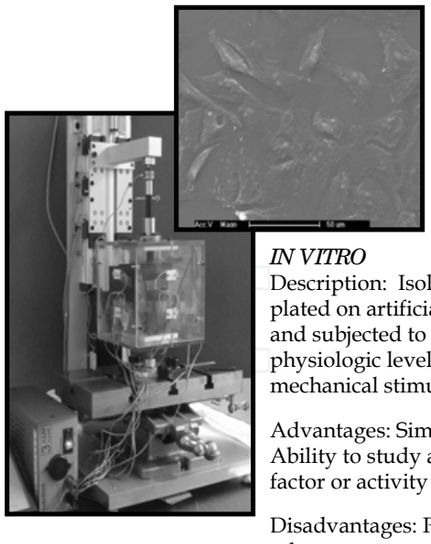


Fig. 2. Once a basic platform is developed, biomechanics work reduces to adequately developing fixtures and testing protocols to test the tissue. Here simple platens utilize a pair of swivel assemblies to ensure loading across the specimen faces regardless of parallelism.

involved and must consider not only the loading apparatus, but also the environment which needs to be held at physiologic conditions. For example, cells need to be tested in a hydrated environment while controlling for variables such as temperature, pH, osmolarity and medium content.

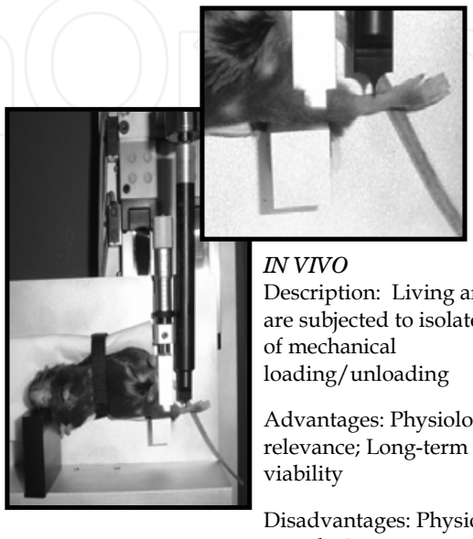
In mechanotransduction, researchers are interested in stimulating bone cells (directly and indirectly) to study the effects of the stimulation on factors such as message (mRNA) and protein production. These models vary greatly in the level of complexity but the two common types of mechanotransduction models are *in vitro* and *in vivo* systems. We will introduce the reader to the idea of mechanotransduction modeling by introducing the *in vitro* and *in vivo* systems and then we will focus on the development of a new approach – *ex vivo*, or biomimetic modeling using an organ culture system. Figure 3 illustrates these types of mechanotransduction systems.



IN VITRO
Description: Isolated cells are plated on artificial substrates and subjected to proposed physiologic levels of mechanical stimulation

Advantages: Simplicity; Ability to study an isolated factor or activity

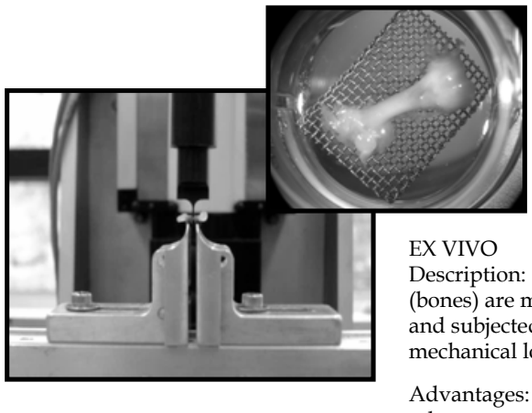
Disadvantages: Physiologic relevance



IN VIVO
Description: Living animals are subjected to isolated bouts of mechanical loading/unloading

Advantages: Physiologic relevance; Long-term study viability

Disadvantages: Physiologic complexity



EX VIVO
Description: Whole organs (bones) are maintained in culture and subjected to isolated bouts of mechanical loading

Advantages: Physiologic relevance over in vitro; Physiologic simplicity over in vivo

Disadvantages: Dying system; Not widely accepted as a mechanotransduction model

Fig. 3. Mechanotransduction research is commonly conducted with *in vitro* and *in vivo* systems. A third model, the organ culture, or *ex vivo* model may also prove beneficial to increase the physiologic relevance over *in vitro* systems while reducing the complexity of the *in vivo* models.

In *in vitro* mechanotransduction work, isolated cells are subjected to mechanical stimulation. As such, it is important that the stimulation/loading mode be physiologically relevant. That is, cells in the experimental system should be stimulated in a manner that corresponds to the living system. In recent years, one of the more physiologically acceptable modes of bone cell stimulation to emerge has been fluid shear (Piekarski and Munro, 1977; Reich, et al., 1990; Weinbaum, et al., 1994; Hung, et al., 1995; Hung, et al., 1996; Owan, et al., 1997). When one

considers the unique environment of particularly the osteocyte, this loading mode becomes evident. That is, osteocytes are mature bone cells that share a common mesenchymal lineage with the osteoblast, the principal bone-forming cell in the body. In fact, the osteocyte is considered an 'aged' osteoblast that in the process of forming bone became walled off in the mineralizing tissue. As the bone-forming osteoblast becomes encased in the bone matrix and transitions to an osteocyte, it takes on a highly dendritic morphology accompanied by structural changes to accompany the reduced need for protein production. A cartoon of the osteocyte environment is shown in Figure 4.

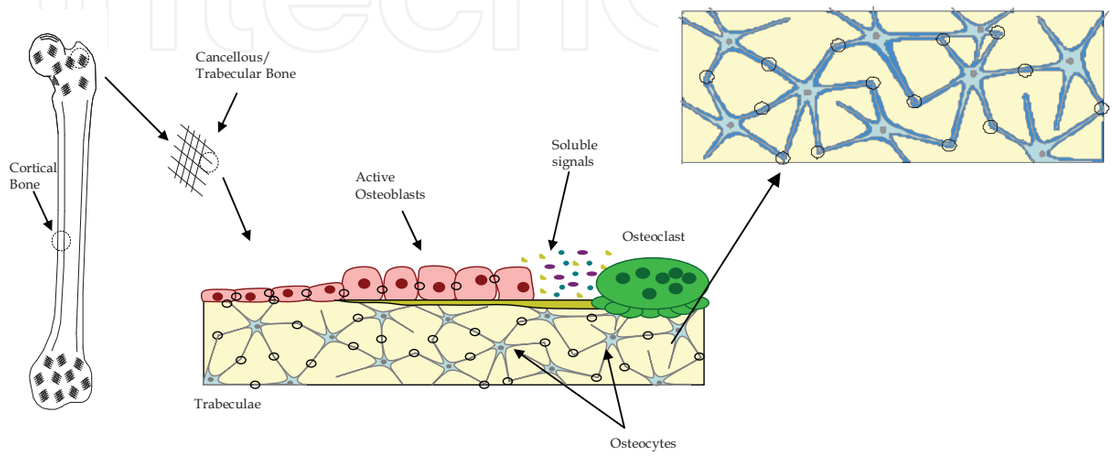


Fig. 4. Osteocytes are former osteoblasts that became encased in the bone mineral during bone formation. Within the matrix, the osteocytes are physically linked to each other via gap junctions in a fluid-bathed lacunocanalicular network. The gap junctions, which also exist between osteoblasts and themselves and osteoblasts and osteocytes (black circles) enable the cells to 'communicate' and share small molecules and ions.

The osteocytes are housed in lacunae, or lenticular shaped cavities that are connected via canaliculi, or little canals. The lacunocanalicular system enables the processes of neighboring osteocytes to physically link while the interstitial fluid found throughout the network bathes the cells and enables important metabolic regulation (Cowin, 1999; Wang, et al., 2004). Given the physical system, what is created is a network of fluid bathed bone cells in a mineralized matrix that is flexed upon loading. As such, the osteocyte is positioned to be a key player in bone mechanoregulation (Bonewald, 2007). During the repetitive loading and unloading of the tissue that occurs during walking, the shifts in the load cause corresponding shifts in pressure gradients that force the fluid from regions of higher pressure to lower pressure. With the rhythmic repetition of walking, the osteocytes are subjected to an oscillatory (not necessarily sinusoidal) fluid shear stress generated by the directional shift in interstitial fluid flow (Piekarski and Munro, 1977; Jacobs, et al., 1998).
One way to generate this loading mode is with the system shown in Figure 5. Here, a parallel plate flow chamber is used in conjunction with a loading platform to deliver physiologic levels of shear stress to cells in monolayer (2-25 dyne/cm^2) via medium-filled glass syringes (Frangos, et al., 1985; Frangos, et al., 1988; Jacobs, et al., 1998). The base of the syringe is anchored to the base of the loading platform while the syringe plunger is attached to the moving slide. Tubing attached to the syringe tip and connected to the inlet of the parallel plate chamber connects the chamber to the platform motion. The parallel plate flow

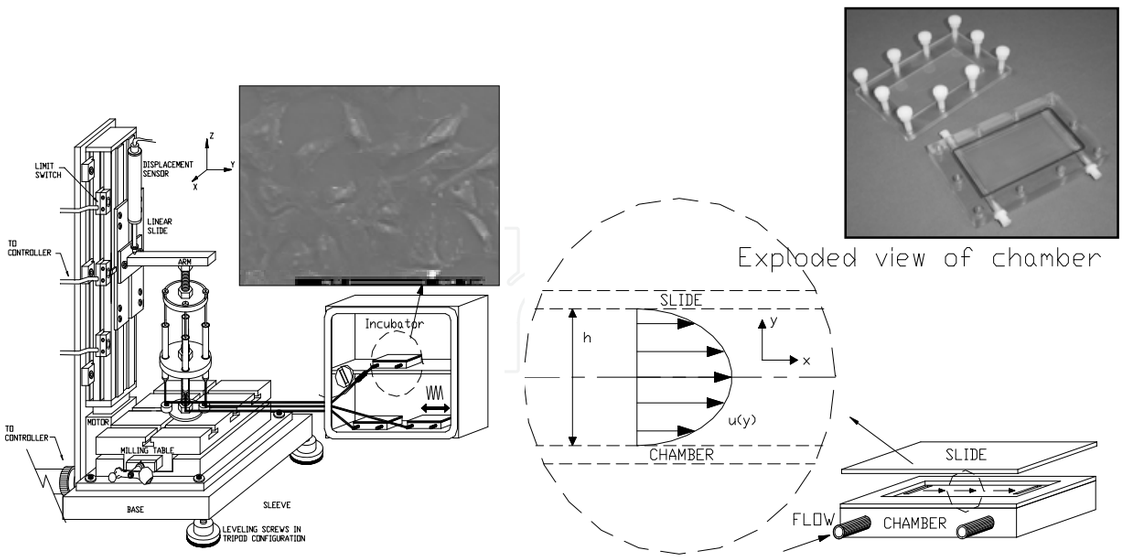


Fig. 5. (a) Syringe assembly used to subject bone cells in a parallel plate flow chamber to physiologic levels of fluid shear (modified from the setup of Jacobs, et al., 1998).

chamber is a polycarbonate chamber with recessed base and slits connected to the channels for the inlet and outlet flows. The cells are seeded on a glass microscope slide that when inverted on the plate forms the lid of the chamber and closes off the fixed volume of the flow chamber. A gasketed lid screwed to the top of the system holds the assembly together and prevents fluid leaks. This system has readily become an accepted method of fluid shear stimulation with fluid shear stress defined by the equation:

$$\tau_w = \left(\frac{6\mu Q}{bh^2} \right)$$

where μ is the viscosity of the fluid; Q is the flow rate; and, b and h represent chamber dimensions of base and height, respectively (Hung, et al., 1985). In *in vitro* stimulation studies, the cells are subjected to the isolated stimulation and depending upon the activity being studied, the cells, supernatant, or cells and supernatant may be collected and analyzed. For example, supernatant may be collected and analyzed for soluble factors released by the cells and the cells may be collected for mRNA analysis or used to normalize the volume of supernatant activity. Time courses are not uncommon and the effect of stimulation over a pre-defined time period may be assessed. It is important in the experimental design to determine the activity to be studied. Whereas soluble activity may be collected over a short duration, the study of mRNA requires a longer time course and sterility issues with the cell maintenance in culture post-flow, as well as flow system sterility are critical.

From an engineering perspective it is important to understand the limitations of this system. As often occurs, the parallel plate flow systems are considered highly characterized and bone scientists have proposed that chamber dimensions and flow properties that yield shear stresses on the order of 5-25 dyne/cm² are physiologic for bone cells. Attached as an Appendix is a derivation of the shear stress in the parallel plate flow chamber. What is important to note is that the derivation is based on the dimensions of the flow chamber

without allowance for the cells, or how the flow at the cellular level might be affected by the cell size, cell adhesion, cell density, cell properties (membrane viscoelasticity), etc.. In addition, developing flow around the slits is not acknowledged and what is derived is an equation for the 'average' shear stress in the empty chamber of known dimensions. These slides hold relatively large volumes of cells and the average response (even when normalized to cell protein) does not yield particularly valuable insights into individual and small cell subpopulation responses.

Another concern when using these systems is to subject the appropriate bone cell type to the appropriate stimulus. For example, there are a number of studies that subject osteoblasts to fluid shear. While osteoblasts (primary and immortalized cell lines) have been readily available for several decades, the osteocytic cell model is much more difficult to primary harvest given the encasement in the mineralized matrix and the current standard immortalized line (MLO-Y4) is relatively new, by comparison to the osteoblast models (Kato, et al., 1997). As such, osteoblasts were subjected to fluid shear under the justification that they mimicked 'young' osteocytes. Although osteoblasts respond to fluid shear levels (You, et al., 2000) information from these types of studies may be very productive in the development of a mechanically loaded bioreactor where the justification of a desired response of the cell to an applied stimulus is less critical than obtaining the desired response, studies conducted to determine underlying mechanisms of osteoblast responsiveness to physiologic stimulation should strive to model an appropriate stimulus. The system illustrated in Figure 6 was developed to apply a physiologic (global level) substrate deformation to surface-residing osteoblasts. Again, the goal was not to determine the most stimulatory loading mode, but to stress the need for physiologically-appropriate systems which mimic the native loading environment (Saunders, et al., 2006).

For these studies, cells were seeded on tissue culture plastic (polystyrene) slides and subjected to three-point bending. The bending subjected the slides to a physiologic strain (maximum 3500 microstrain) verified with strain gauges placed on the underside of the slide. Direct cell strain was not quantified. In addition, an environmental chamber was developed from polycarbonate and heated with microheaters. The environmental chamber held a medium-filled reservoir that enabled the cells to be submerged during testing and pH and humidity were controlled. As shown in Figure 6, three-point bending was selected over four-point bending to maintain a combination of bending and shear throughout the field of loading. This was in contrast to the four-point bending scenario in which pure bending resulted between the inner two points of contact. Given the previous response of the osteoblasts to shear, three-point bending was used to subject cells to a physiologic environment of primary substrate deformation and secondary fluid shear. Strain gradient changed as a function of contact placement, but a linear relationship was assumed and verified with preliminary strain gauge testing on the substrate. Regardless of stimulus, the need to maintain cell hydration results in the presence of secondary fluid shear forces that can confound findings.

In vitro mechanotransduction systems have also incorporated co-culture models. In these systems it is acknowledged that the interaction between the various cells types is critical and devices to load the systems in a tandem model have been developed. Shown in Figure 7, is a device in which osteoblasts and osteocytes are co-cultured (Taylor, et al, 2007). The focus of this work was to study the mechanically-stimulated osteocytic effects on osteoblasts in physical contact with the osteocytes via gap junctions, but effectively isolated from the stimulation. In this system, osteocytes are grown on one side of a porous mesh and

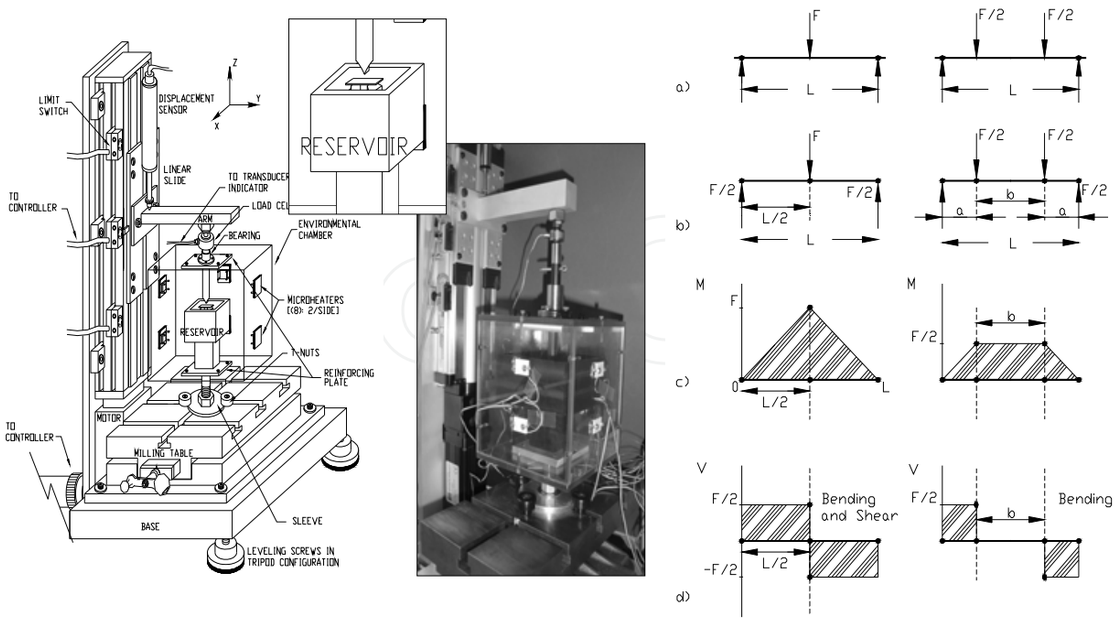


Fig. 6. Substrate deformation assembly used to subject osteoblasts to physiologic levels of bending (and secondary fluid shear). The environmental chamber was developed to maintain temperature; pH and humidity were also regulated. Cells in these systems are plated in monolayer on a synthetic substrate and subjected to stimulation.

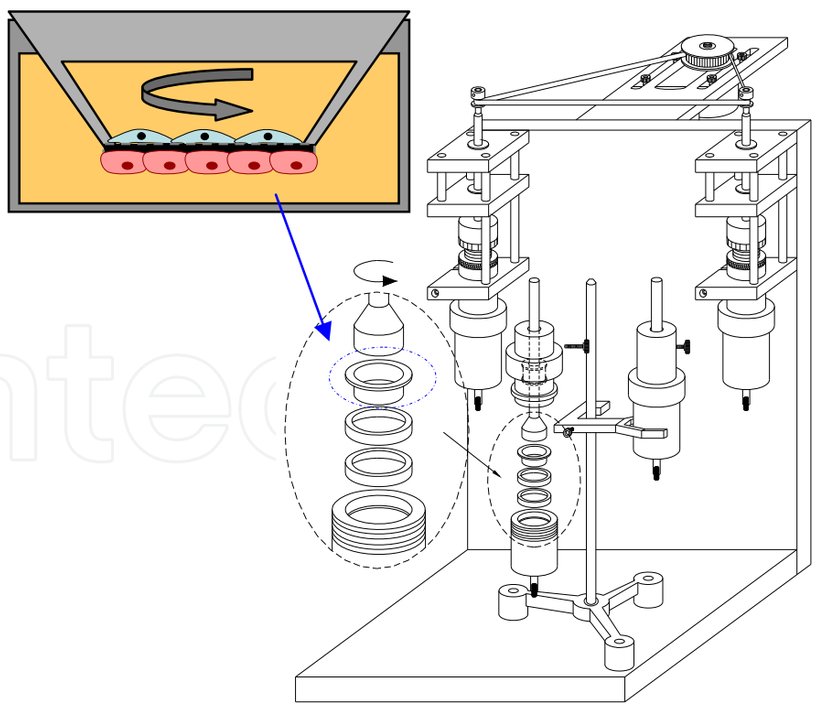


Fig. 7. Rotary stimulatory system to study osteocyte/osteoblast interactions via gap junctions. Stimulated systems are compared to controls placed in similar chambers (ring stand) without the load.

osteoblasts are grown on the other. The well containing the co-cultured mesh is placed in the loading device and a programmable motor enables a belt-driven assembly to apply fluid shear to the osteocytes in culture via a rotating disk. The cell density and the thickness of the mesh effectively ensures that the only physical contact the cell types have with each other is via the gap junction channels that grow through the porous mesh. The system is then utilized to study the indirect effect of mechanical stimulation (via fluid shear) on osteoblastic activity, as well as the importance of osteocytes (and gap junctions) in transducing a mechanical signal. These types of models are important not only for the basic science value of the findings, but because they represent a necessary trend toward increasing the physiologic complexity (and biomimicry) of the isolated *in vitro* environment.
These are just three examples of *in vitro* stimulation systems. And while others exist (Ajubi, et al., 1996; Bottlang, et al., 1997; Ziambaras, et al., 1998; Brown, et al., 1998; Brown, 2000), they all have the same purpose. *In vitro* mechanotransduction models strive to understand the cellular pathways and mechanisms by which bone cells respond to mechanical stimulation. Advantages of these systems include their simplicity and the ability to isolate a particular factor for further study. Disadvantages include their simplicity which makes relevant comparisons to the physiologic models difficult, at best. There are also cellular concerns that should at least be acknowledged but are beyond the focus of this work. For example, it is not clear (and highly improbable) if the extrinsic load translates directly to an intrinsic load and the cell 'sees' the identical global load. Furthermore disparities within and between cell models (primary sources and immortalized lines) and the day to day changes that occur with cells further complicates comparisons. As such, results from cellular studies generally are presented with respect to a particular osteoblastic cell line or cell lines with emphasis on maintaining appropriate culture conditions and low passage numbers for immortalized cells or noting digestion numbers and passage numbers (generally not more than 3-4) for primary isolations (Sorkin, et al., 2004).
In *in vivo* models, living animals are subjected to loading environments under highly regulated conditions. In these systems, animals may be subjected to sub-physiologic (underload), physiologic or supra-physiologic (overload) loading environments and the isolated effects may be studied for an extended period of time. For example, these models include loading to simulate use and overuse and unloading to simulate weightlessness and bed rest/paralysis. For loading studies bones may be loaded by training the animals (generally rodents) to stand on their hindlimbs while increasing the weight on their backs. A common way to accomplish this is to train the animals in cages with a live floor to send an electric shock as negative reinforcement and food as positive reinforcement (Buhl, et al., 2001). Another method to apply load to *in vivo* models is to anesthetize the animals and put them in a device that loads the limbs in a desired manner (Turner, et al., 1991; Hillam and Skerry, 1995; Gross, et al., 2002). To this end, the in-house platform was used and a loading fixture developed that enabled the application of a concentrated, cantilevered load to rodent tibiae, Figure 8. In addition, the platform was designed to rotate such that the limb could be loaded in the anteroposterior or mediolateral orientation. For torsion and *in vivo* stimulation studies the movable base of the milling machine table makes it trivial to align the specimens in the loading machine for testing. Unlike standard commercial testing systems, the planar motion provided by the milling machine table of the in-house platform enables the attached fixtures to be dialed-in either front-to-back or side-to-side then locked down for testing. Advantages of *in vivo* systems include their physiologic relevance and the ability to study the outcome of the cellular stimulation (bone formation/resorption) over a longer period of

time. Disadvantages include the physiologic complexity that makes it difficult to discern the direct response of the cell to the stimulation and the loss in sensitivity that has been observed in using these systems with repeated loading.

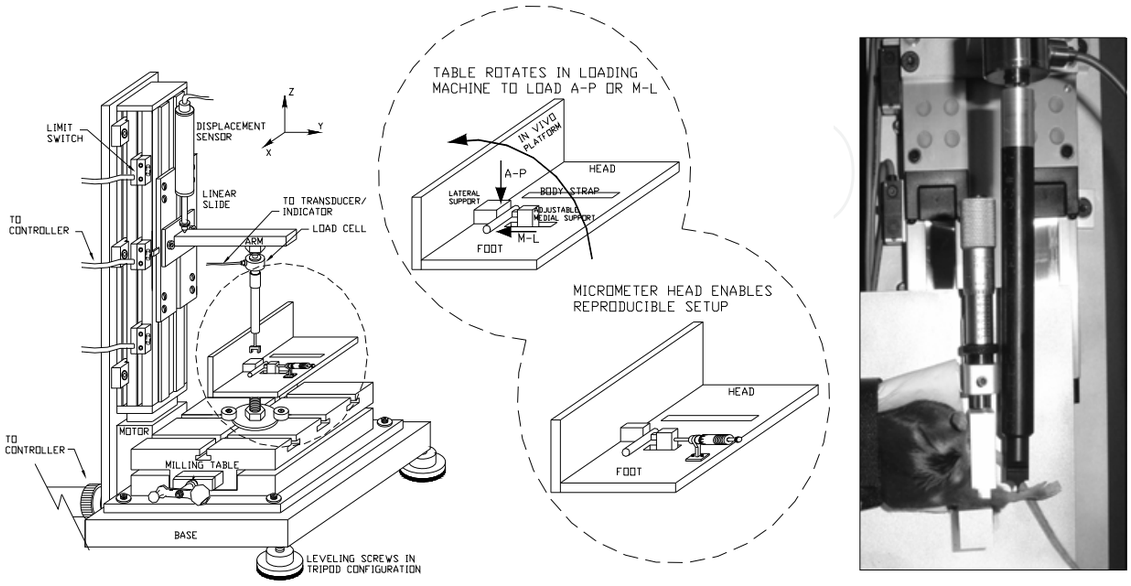


Fig. 8. Bending assembly for *in vivo* stimulation studies. The platform rotates to load rodent tibiae in the anteroposterior (A-P; front-to-back) or mediolateral (M-L; side-to-side) orientations. A cantilevered loading is produced in which the proximal end of the tibia is held in place and the distal end is cyclically stimulated (modified from the setup of Gross, et al., 2002).

As with any type of research model, there are advantages and disadvantages to using each system and it is important for the researcher to appreciate this such that they may utilize each system to their full advantage. It is also important to realize that it takes time for research models to be proven valid and acceptable in a given area, but there will always be inherent flaws in any model. For example, the use of osteoblastic cell lines in mechanotransduction is well accepted. However, the new investigator may be surprised to learn that in many cases the cells that are modeled as osteoblastic are not derived from the bone directly, but from bone cancers. For example the long-used ROS 17/2.8 immortalized cell line is, as its name implies a Rat Osteoblastic Sarcoma cell line derived from bone tumor since tumor cells do not undergo terminal differentiation, but continue to proliferate. Additional immortalized lines derived from tumorigenic sources include the rat UMR-106 and UMR-108 lines (Forrest, et al., 1985) and the human U-2 OS and MG-63 lines (Ponten and Saksela, 1967; Billiau, et al., 1977). Furthermore, it has been recognized that some nontumorigenic cell lines, such as the mouse MC3T3-E1 line loses its osteogenic properties with time in culture (Takahashi, et al. 2002). In addition, the ultimate goal will always be the use of these systems to address, regardless of origin, the human condition. Osteoclast (bone-resorbing cells) models that have been widely studied include the chick model; however, the chick osteoclast, in contrast to the human osteoclast, was originally thought not to possess the calcitonin receptor (Nicholson, et al., 1987) and later determined to possess detectable levels under dietary modification (Gay, 1991). While this is not to imply

that these research models are irrelevant, the intent is to show that all models have limitations and perfect systems do not exist. The goal of developing a new model is to look at the strengths and limitations of the available systems and weigh the benefits of a new model against those of the accepted standards. With this in mind, we set out to develop an *ex vivo* (organ culture) model for bone mechanotransduction that we feel may fill a specific niche in the research field by providing a system that is biomimetic.

Organ culture models are not a new concept (Fell, 1972; Boyd, 2005). The earliest organ culture experiments date back to 1859 when Valpian attempted to maintain frog embryo tail fragments in water (Fell, 1972). In 1897 Born and Loeb used this approach to maintain small fragments of rabbit organs in serum. In 1912 McWhorter and Whipple successfully used the organ culture approach to maintain entire avian and mammalian embryos in blood plasma to study the development of chick blastoderms (Fell, 1972). A pioneer in the field of organ culture modeling, Dame Honor B Fell considered the first true organ culture to be the work of David Thompson (1914). Thompson used a hanging drop method to explant toes, feather germs, tongue tips, optic lenses and tail buds from embryonic chicks into plasma to study their development (Balls, 1976). Fell's research was critical to the use of organ culture in the musculoskeletal field (Fell, et al., 1976). She began her organ culture research in 1924 when she studied the development of undifferentiated limb buds of chick embryos. She found that when cartilaginous bone from the limb of 5-6 day old chick embryos were kept in culture, they continued to grow and develop histologically and anatomically. Later it was found that these bones gave essentially the same response to certain vitamins and hormones in culture as they did *in vivo*. Additional pivotal organ culture work in the bone field was conducted by Glucksmann who showed that bone could respond to mechanical stimulation. Glucksmann used a clever culture technique to show that bones grown in small culture dishes were, as they grew, subjected to stresses from the culture dish walls that resulted in ossified tissue aligning along directions of tensile stress (Glucksmann, 1942). Organ culture systems enjoy a wide use in research (Stepita-Klauco and Dolezalova, 1968; Barrett and Trump, 1978; Jubb, 1979; Merrilees and Scott, 1982; Weiss, et al., 1988; Wetzel and Salpeter, 1991; Ishizeki, et al., 1995; Merrick, et al., 1997; Voisard, et al., 1999; Takezawa, et al., 2000; Del Rizzo, et al., 2001; Swanson, et al., 2002; Lyubimov and Gotleib, 2004) but their presence in the bone field is much less prevalent. Historically the use of organ culture models in bone research has been as an experimental system to study the effects of resorptive agents on bone (Raisz, 1965; Raisz and Niemann, 1967; Reynolds, 1976; Murrills, 1996). To be useful for biomimetic mechanotransduction modeling, not only must the model retain its viability in culture, but it must be able to respond to mechanical stimulation with a response indicative of an osteogenic event. In addition, it is important to note that there are systems in which bone explants/rudiments are maintained quite successfully for significant periods of time (Jones, et al., 2003; Takai, et al., 2004; Davies, et al., 2006; Chan, et al., 2009). One such system, the 'Zetos' system is a clever perfusion chamber that enables trabecular cores (from bovine sources) to be kept viable for extended periods of time (Jones, et al., 2003; Davies, et al., 2006). While the value of this system is significant, our goal was to develop an organ culture model of an intact bone. In addition to incorporating both cortical and trabecular bone, the whole organ culture model aimed to preserve the integrity of the periosteum.

The major drawback with the use of the organ culture is that it is a dying tissue at the time of harvest and it remains to be determined if these systems can provide acceptable models of bone cell mechanotransduction pathways and mechanisms. Indeed, they have shown promise in short-term studies on the order of hours (Zaman, et al., 1992; Dallas, et al., 1993).

If proven acceptable, these systems could be used to study isolated loading effects that would increase the physiologic relevance over *in vitro* systems (adding biomimicry) while reducing the complexity of the *in vivo* systems (ie, eliminating systemic effects). For the concept of biomimetic mechanotransduction modeling to be validated it is necessary to show that the models remain viable in culture, continue to grow and respond to brief bouts of physiologic stimulation. Ideally it would also be advantageous to demonstrate that these models can simulate relevant clinical systems and provide an additional tool for bone scientists.

Our organ culture work initially employed a rodent long bone model. The femur was chosen for its routine subjection to repetitive loads (walking). Neonatal rodents given the increased porosity of this model over a fully calcified adult system were selected. Rodents were chosen to enable future molecular analysis and rats (Wistar), over mice, were chosen for convenience given the larger size of the bones. Work to date has used neonates ranging from 2-5 days old with much of the repetitive loading studies conducted in 5 day old models given their reduced fragility at this age. Bones were isolated using blunt finger dissection to preserve the periosteum and maintained in an incubator (5% CO_2) in BGJb medium supplemented with 15% fetal bovine serum and 2% penicillin/streptomycin as previously recommended (Meghji, et al., 1998; Garrett, 2003; Saunders, et al., 2010). The initial systems were maintained in standard organ culture medium and passive diffusion provided the only source of transport. Bones were cultured in 12 well culture dishes on stainless steel mesh inserts such that 2 ml of medium covered the bone shafts while exposing a small region of the cartilaginous ends to the air to maintain the bones at a liquid/gas interface. Medium was topped off daily to keep the bone shafts submerged and completely replaced every 3 days. Medium was warmed to 37 °C before adding to the cultures to avoid shocking the bones. In addition, with the exception of viability studies where control bones were taken immediately post-harvest (maximum viability), all other controls were taken at 24 hr for growth studies. The 24 hr period was necessary to allow the bones to equilibrate to the culture conditions. (Meghji, 1998) Furthermore, in all cases, the initial medium change was 24 hr post-harvest to remove any deleterious soluble effects of the harvest on the cultures. For example prostaglandin levels increase as a result of trauma (dissection) and prostaglandins have a direct effect on bone formation/resorption (Jee, et al., 1985; Imamura, et al., 1990; Klein-Nulend, et al., 1997; Hagino, et al., 2005).

It is important to keep in mind that the benefit of using the organ culture system is that the cells are stimulated in their native environment and the response of the cells at the tissue level may be quantified. However, the tissue level response is not instantaneous and the culture must stay viable long enough for the stimulation at the tissue level to be observed. Furthermore, much of the mechanotransduction field is concerned with understanding the response to physiologic levels of loading which incorporate modest magnitudes and short durations. That is, to be effective it is necessary to demonstrate that the organ culture model can respond to a brief physiologic bout of loading and can maintain viability during this period. Pilot studies (not shown) suggested that 1 wk was an appropriate culture time to anticipate a response in these systems, and as such, was used as a starting time point for the organ culture studies.

To assess the viability of the cultures, standard hematoxylin and eosin (H&E) and lactate dehydrogenase (LDH) staining were completed. H&E staining, as shown in Figure 9 is beneficial to show that there are osteocytes present in the lacunae. In Figure 9, the midshaft bone section, taken from a 2 day old neonate and maintained in culture for 2 wk indicates

that in the midshaft of the femur, (sectioned between 5-10 microns thick) osteocytes are abundant in the lacunae. However, the H&E stain does not indicate if the cells are viable, it only verifies their presence in the matrix at the time of fixation. To quantify osteocyte viability, LDH staining was completed.

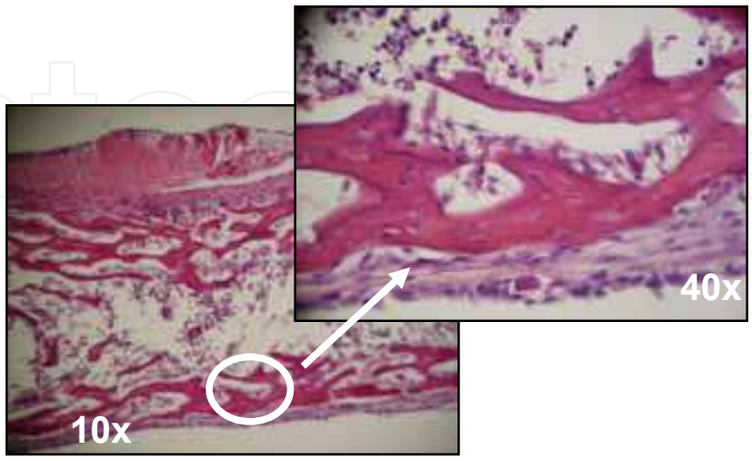


Fig. 9. H&E staining on femurs following 2wk of culture supported osteocyte viability as shown by the majority of lacunae showing osteocyte bodies.

Just as humans require energy to complete any function, cells require energy for all their normal activities. Lactate dehydrogenase is an enzyme involved in energy conversion and since only living cells require energy, cells staining positive for LDH are viable (alive) while cells not staining positive are non-viable (dead). In cryosectioned slices, matrix-embedded osteocytes were quantified for viability (Wong, et al., 1982). To determine viability as a function of time in culture, bone pairs were randomly analyzed with 1 femur stained immediately at the time of harvest and the contralateral control was stained following 1 or 2 wk in culture. The 2 wk time point was completed to assess the viability of the cultures passed the desired time point of 1 wk. All lacunae in a given section were counted and a minimum of 12 sections were counted for each time point. As shown in Figure 10, approximately 93% of the osteocytes were viable at the time of harvest while 70% of the osteocytes were viable at 1 wk and 42% were viable at the 2 wk time point (data not shown) (Saunders, et al., 2010). Given that no additional efforts were made to enhance viability (eg, perfusion or topical additives), the viability counts were adequate. Computational permeability modeling proposed by Botchwey and Beno supported these findings (Botchwey, et al., 2003a; Botchwey, et al., 2003b; Beno, et al., 2006). It is anticipated that these viability results could be improved. However, it must be kept in mind that the intent is to determine the usefulness of this culture model and as such, perfusion or topical additives should aid viability without being overly stimulatory to the culture. The objective is to be able to discern the effect of the isolated loading and the efforts to improve viability should not confound the effects of the loading.

To assess growth in culture, bones taken from 2 day old neonates were analyzed for changes in length, weight and mass distribution. Presented here are results during the 1 wk culture period. The control limb was analyzed 24 hr after harvest. For length comparisons, overall femur length, shaft length and shaft diameter were determined. Bones were digitally imaged at the corresponding time point and NIHImage software (ImageJ) was used to

determine measurements. Three measurements for each location were determined and averaged. Femur length increased 10.3%; shaft length increased 6.3%; and, shaft diameter increased 6.5% in culture. All increases were statistically significant (Saunders, et al., 2010), Figure 11.

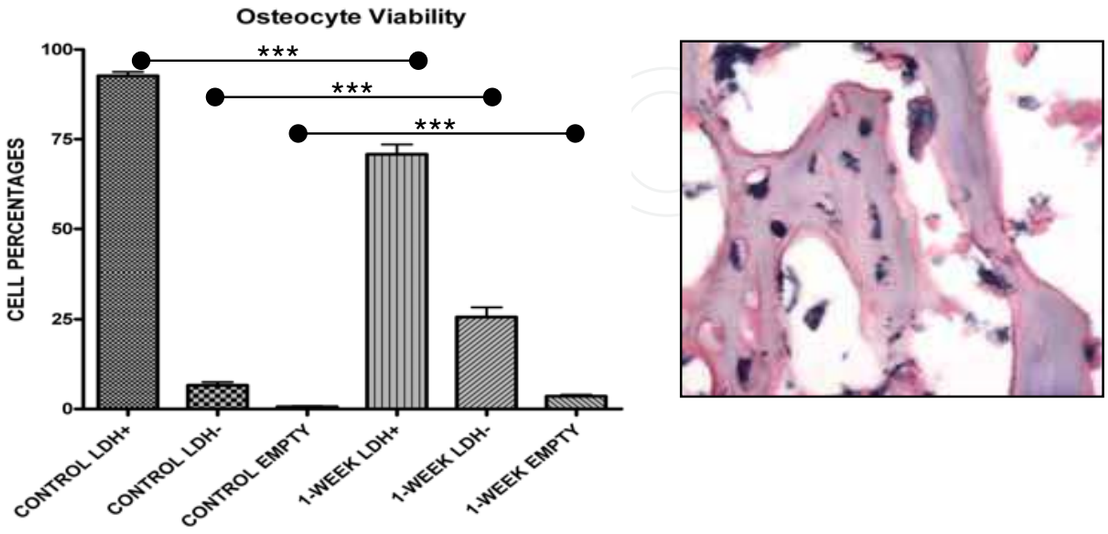


Fig. 10. Osteocyte viability was quantified with LDH stains and manual cell counts. Osteocyte viability at 1 wk of culture was 70 % as determined by the number of osteocytes staining positive for LDH.

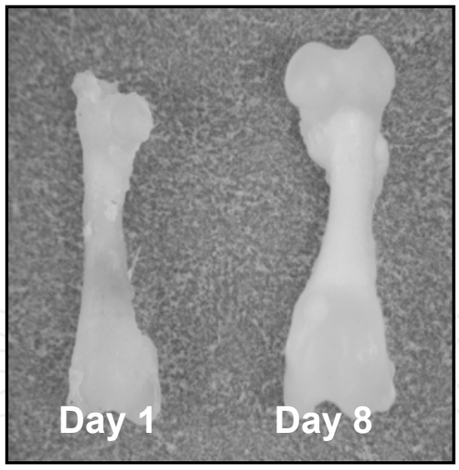


Fig. 11. Femur growth over the 1 wk culture period was significant.

Weight changes in culture were quantified using previously established techniques (Mikic, et al., 2002). Wet, dry and ash weights were obtained and represent, to a crude degree, the content of the bone. Wet weight, as the name implies represents the natural culture weight (including water content) of the bone and is obtained after removal of any free medium from the bone. Dry weight is obtained following defatting of the bones in acetone and thoroughly drying under low heat. Ash weight represents the mineral content and is obtained following burning the bones at high temperatures (600 °C) for an extended period

of time (6 hr). For *ex vivo* models, ash weight is an important parameter to assess changes in bone mineral development as a function of culture period and environment. Weights continued to increase with the 1 wk culture period. Specifically, wet weight increased 85.2% in culture; dry weight increased 49.5%; and, ash weight increased 57.5%. All increases were statistically significant (Saunders, et al., 2010). The increases in wet weights revealed that the bulk of the weight in these cultures, not unexpectedly, was the result of tissue hydration and the cartilaginous material. However, the increases in the ash weights over the 1 wk culture period suggests that the bone was also increasing in mineralization, which is what would be desirable in a mechanotransduction system.

The distribution of the mass of bone reveals useful information about its strength and its ability to resist loading and deflections in given orientations. For example, the polar moment of inertia provides a measure of the ability of the specimen to resist torsion. To assess areal properties, bone shafts were embedded in polymethylmethacrylate (PMMA) bone cement and sectioned on a diamond saw following dehydration in alcohol and digitally imaged. Moments of inertia increased 40.9% and 34.9% in culture assuming an elliptical cross-section, Figure 12. Polar moment of inertia increased 37.7% while cortical area increased 16.6%. All areal changes were statistically significant.

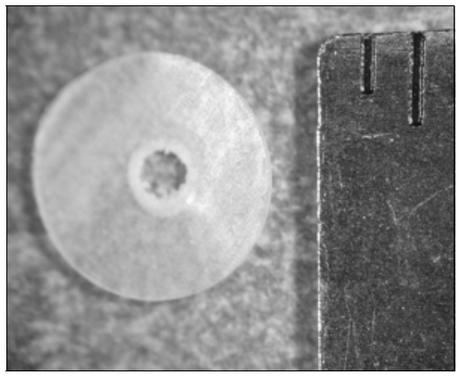


Fig. 12. Typical cross-section of neonatal femur. Ruler ticks represent 1.6 mm increments.

While these results taken together clearly show that the bones are viable over the 1 wk culture period and continue to grow, these measurements are rather rudimentary. To strengthen the premise that organ cultures may be used as biomimetic models of mechanotransduction, additional, more accurate techniques have also been employed. Whereas the LDH stain indicates cell, particularly osteocyte, viability, the overall organ viability was also assessed with micro computed tomography, or microCT. In microCT studies, femurs from 5-day old neonates were harvested and maintained in culture for 1 (n=9), 2 (n=9) or 4 wk (n=10) of culture while the contralateral control was analyzed 24 hr post-harvest. Bones were analyzed using a MicroCT-40 scanner (Scanco Medical, Basserdorf, Switzerland) using source settings of 55 kV, 145 μA and high resolution. A midshaft analysis was performed, producing 50, 2048 x 2048 2D axial slices in the midshaft of each bone (0.4 mm). Inertial, areal, volumetric and density measurements were determined. Cultured bones at 1, 2 and 4 wk were normalized to their contralateral controls and compared. Shown in Figure 13 are results for the 1, 2 and 4 wk culture comparisons. Overall, all properties were found to significantly increase over the 1 wk culture period ($p<0.001$) and normalized 2 and 4 wk comparisons are reported as percent decreases relative to the 1 wk

observations. Specifically, polar moments of inertia (pMOI) decreased 23.4% ($p<0.05$) and 36.5% ($p<0.001$) over the 2 and 4 wk culture periods, respectively. Resistance to bending (assuming an elliptical cross-section) decreased 25.9% (Imax/Cmax) ($p<0.05$) and 24.0% (Imin/Cmin) ($p<0.05$) over the 2 wk culture period and 39.3% (Imax/Cmax) ($p<0.001$) and 38.6% (Imin/Cmin) ($p<0.001$) over the 4 wk culture period. Density decreased 41.6% ($p<0.05$) and 53.0% ($p<0.001$) over the 2 and 4 wk culture periods, respectively. These findings, not surprisingly, suggest that the organ culture models, as maintained here, are most effective if used for short term studies (≤ 1 wk) and that while the bones at 2 wk of culture are more dense and stronger than their counterparts at 24 hr post-harvest, their loss of viability is significant given the comparison to the 1 wk bones. Four week results indicate a relatively dead culture that in many cases was approximately equivalent to the 24 hr controls.

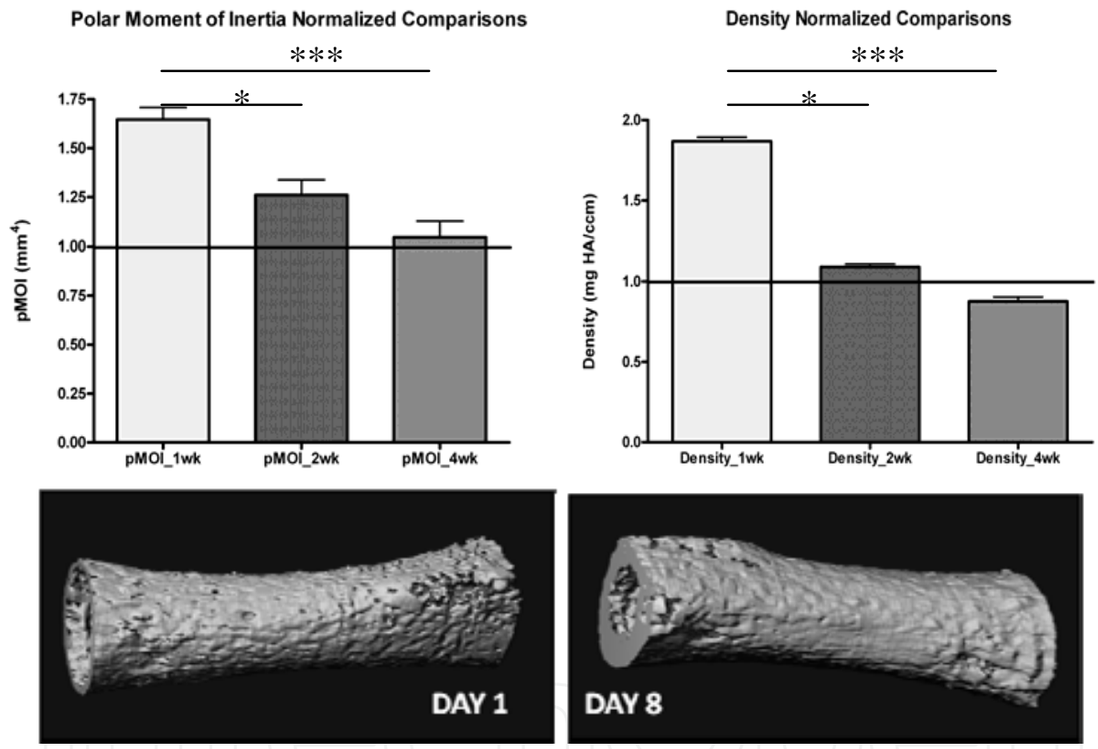


Fig. 13. Results from microCT analysis indicate significant increases in bone shaft quantity (moment of inertia) and quality (density) at 1 wk of culture that significantly degrades by 2 wk of culture.

Once it was determined that the bones could remain viable in culture for 1 wk and could continue to grow under the culture conditions, the bones were subjected to an isolated bout of stimulation and the response assessed. A major objective of the organ culture system is to show that it is capable of responding to a brief, isolated load. The reason for this from a research standpoint is to be able to utilize the model to study the immediate response to short-term loading; from a practical standpoint, given the consistency of the cultures it is unlikely that they would survive, i.e. keep their structural integrity, extended bouts of loading.

Gross' group has successfully developed *in vivo* models of mechanotransduction in which the rodent limbs are subjected to short durations of stimulation and the organ culture systems utilized similar loading durations (Gross, et al., 2002). To minimize contamination risk associated with a loading regime over multiple days, the bones were subjected to one bout of loading in which 350 cycles of loading were applied over 5 mins (1.17 Hz). In these experiments, the control bone was maintained in culture for 1 wk without load, while the treated limb was subjected to 350 cycles of loading at a maximum strain of 500 microstrain (minimum of 50 microstrain) 24 hr after harvest. Following stimulation via three-point bending, the bones were returned to culture for 1 wk. Following the 1 wk culture period the control and stimulated bones were mechanically loaded to failure and the structural effect of the loading was quantified. It was determined that the brief bout of loading resulted in a significant increase in stiffness (34.5%) (Saunders, et al., 2010). Failure load was increased 5.8% and displacement was decreased 11.2%. Taken as a whole, the mechanical testing results of an increasing trend in stiffness and failure load with a subsequent decreasing trend in failure displacement are indicative of an increase in strength with the stimulation, or an osteogenic response.

The initial objective of developing a biomimetic organ culture model of bone mechanotransduction has been completed. To date, it has been demonstrated that the neonatal bones may be maintained in culture for periods exceeding 1 wk and that these cultures remain viable, continue to grow and respond to a brief, isolated physiologic bout of stimulation with an increase in mechanical strength. While not an exhaustive analysis, the results of these studies suggest the biomimetic bone mechanotransduction system shows promise. Additional objectives would be to show that these models can be used in studies relating to mechanical load, similarly to *in vitro* and *in vivo* systems. For example, it would be beneficial to show that these systems can respond to topical additives and may be used in a similar way in which the *in vitro* models are used to topically affect a single protein, factor or activity and study the effect. In initial topical studies using the organ culture models, functional communication (gap junction function) was topically inhibited and the effect on mechanical strength during growth in culture was assessed.

Gap junctions are protein channels that enable neighboring cells to physically connect, Figure 14. These channels enable the cells to share molecules and small ions in their interior compartments with each other. As a result, these channels allow the cells to 'communicate'. Gap junctions are abundantly found in bone and osteocytes and osteoblasts are able to communicate with themselves (homotypic coupling) as well as with each other (heterotypic communication). As such, this communication network provides an ideal system of conduits by which cells can coordinate responses to mechanical stimulation (Saunders, et al., 2001; Saunders, et al., 2003). Functional communication may be inhibited with the topical inhibitor 18α-glycyrrhetinic acid (αGA). While inhibiting communication in *in vitro* studies has enabled isolated markers to be studied, *in vivo* models have been hampered by the embryonic lethal nature of the knock-out models (Ewart, et al., 1997). Utilizing the organ culture system, neonatal rat long bones were harvested from 5 day old Wistar rats, digitally photographed, weighed and cultured in standard growth medium supplemented with 30 μM αGA (in DMSO) for 96 hr then replaced with standard growth medium for the remainder of the wk; this process was repeated for an additional 96 hr for the 2 wk culture model. Topically treated femurs were normalized to intact controls. Additional control limbs were treated with an equal concentration of DMSO, normalized to intact controls and compared at 1 and 2 wk of culture. Preliminary results demonstrated that 96 hr in 30 μM

αGA decreased mechanically-induced stiffness 10% following 1wk of culture and 20% following 2 wk of culture in comparison to contralateral controls (Figure 15) illustrating that the topical αGA is effective in inhibiting communication and that this communication contributes to organ culture development.

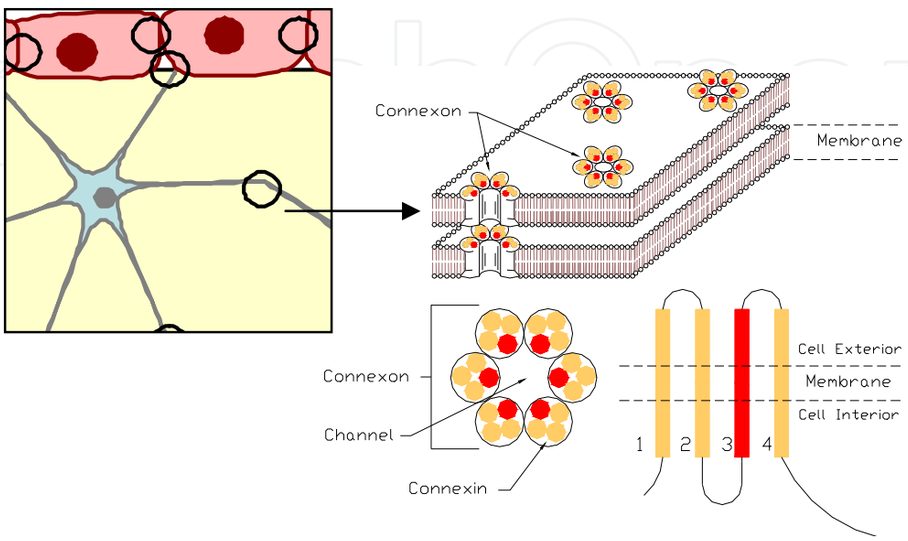


Fig. 14. (Left) Osteocytes communicate with osteoblasts and other osteocytes via gap junctions. (Right) Gap junctions are channels that physically link cell membranes and enable cells to exchange their intracellular contents. Gap junctions are protein channels formed from the union of connexons in adjacent membranes; connexons are comprised of six protein sub-units called connexins named for their molecular weight and abundant in bone cells.

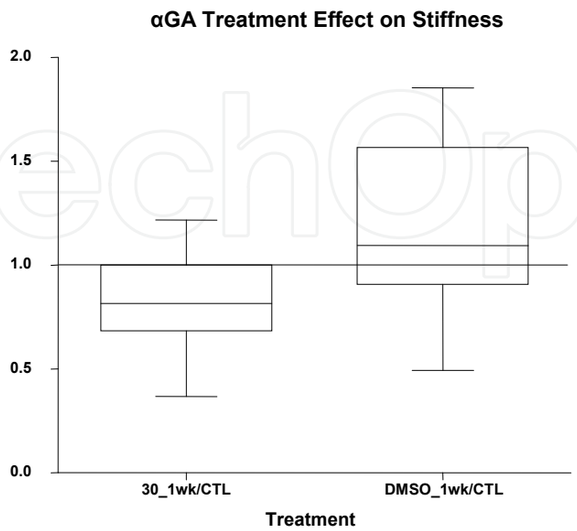


Fig. 15. Decrease in organ culture stiffness with addition of topical gap junction inhibitor.

In addition to being useful for topical studies, the biomimetic models also show promise in preliminary work simulating distraction osteogenesis. Distraction osteogenesis (DO) is a clinical procedure in which bones are subjected to distraction forces that induce new bone formation. While DO has successfully been used to correct orthopaedic and dental deficiencies, DO has also been used to treat mandibular hypoplasia in children associated with Goldenhar's, Nager's and Pierre Robin syndrome (Pruzansky, 1969; Murray, et al., 1979; Lauritzen, et al., 1985). In these cases, DO is performed to correct facial deformities of varying severity and symmetry which can have tremendous psychological benefit. Of more immediate concern, distraction of the mandible can also correct tongue-base airway obstruction and restore respiratory function in neonatal infants as young as 2 weeks of age (Sidman, et al., 20001). However, optimization of the distraction technique is not likely given that the mechanically-induced cellular response of bone has not been elucidated, and the vast majority of clinical applications of DO have been in adult patients. To develop successful loading strategies for DO, quantification of the cellular and tissue responses to loading is required. As such, research addressing the cellular response of bone to distraction in organ culture neonates may help to maximize clinical outcome by optimizing load parameters including magnitude, orientation and duration. A major advantage of the DO technique is that the cellular stimulation induced by distraction can result in sufficient osteogenesis independent of additional bone grafting, and as such offers a significant native improvement over techniques requiring avascular bone grafting. DO, popularized by Gavril Ilizarov in 1951 was originally applied to long bones, and most of the characterization of the approach has been studied in this model system. Considerably less characterization has been conducted in cranial models; the complicated geometry of the jaw in comparison to the femur has been a major factor contributing to this disparity. Thus, using only *in vivo* models with poorly defined distraction has failed to enable the isolated study of the effects of the mechanical loading on cellular response; model system development enabling this would be advantageous.

In essence, DO requires the creation of a pseudo growth plate. The bone is fractured and a distraction device spanning the fracture is used to apply mechanical forces across the fracture to create new bone to fill in the bone gaps. Generally DO involves three phases, the latency phase following an osteotomy/corticotomy where the bone is allowed to begin healing, an active distraction phase in which the bone is actively distracted at a rate generally not exceeding 1 mm/day and a consolidation phase in which the bone is no longer distracted and is allowed to strengthen before the distraction device is removed. In cases of mandibular distraction such as that which occurs in neonates as a result of airway obstruction, the latency phase is not necessary and the bone is pliable enough at this stage to be osteogenic without requiring the initial osteotomy/corticotomy. However, as previously noted, given that little is understood about the response of bone to distraction loading, the procedure often reduces to trial and error. Moreover, in cases of craniofacial deformity correction, symmetry is critical to achieve the desired aesthetics to within fractions of a millimeter. To begin to address DO in organ culture, femur models and linear distraction were utilized. Femurs harvested from 5 day old neonatal rats were subjected to distraction. For loading, a single purpose distraction device was developed given the need for greater accuracy than the in-house loading platform provided and utilized a micrometer head, Figure 16. Utilizing this device, initial studies were conducted in which bones were subjected to 2% strain via linear distraction for 2 hr at 24, 72 and 120 hr of culture. Strain was verified with optical techniques and bones were kept hydrated at all times throughout

the loading period. While distraction is generally performed daily, these initial studies utilized loading every other day to minimize handling and the risk of contamination. At 1 wk the loaded bones were mechanically tested to failure and their no-load contralateral counterparts were used as controls.

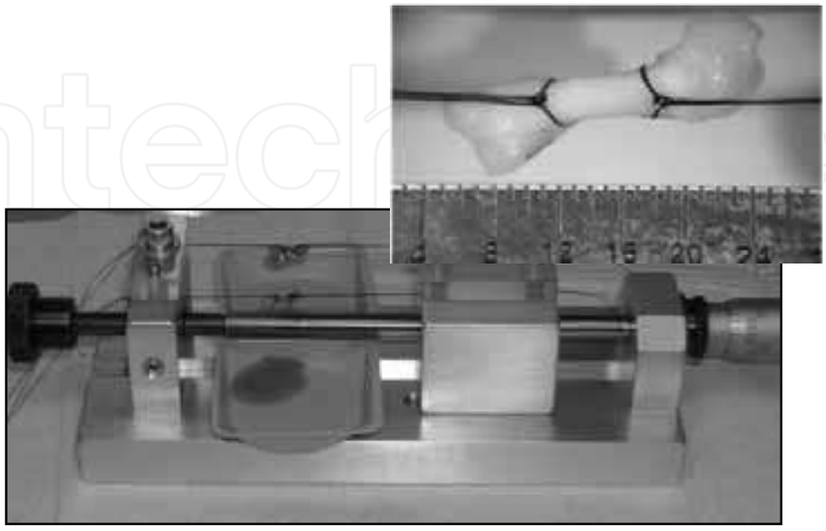


Fig. 16. In-house device used to apply distraction to organ cultures at 2 % bone shaft strain for 2 hr at 24, 72 and 120 hr of culture.

As shown in Figures 17 and 18, bones subjected to 3, 2 hr distraction loads resulted in a significant increase in failure load and stiffness at the 1 wk culture period. Given that the structural integrity of the bones was maintained during the multiple loading bouts and the loaded bones demonstrated an osteogenic response consistent with distraction, the biomimetic models may hold promise in elucidating the pathways and mechanisms by which the bone cells respond under mechanical distraction.

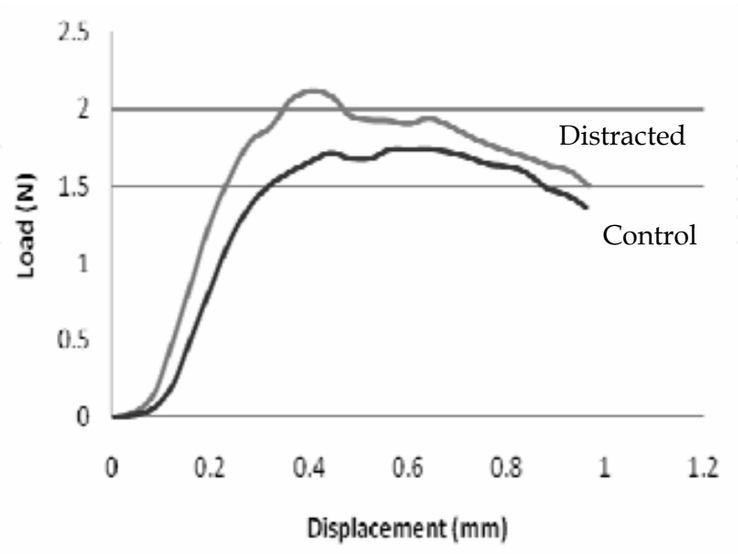


Fig. 17. Typical load-displacement curves for distracted and control bones.

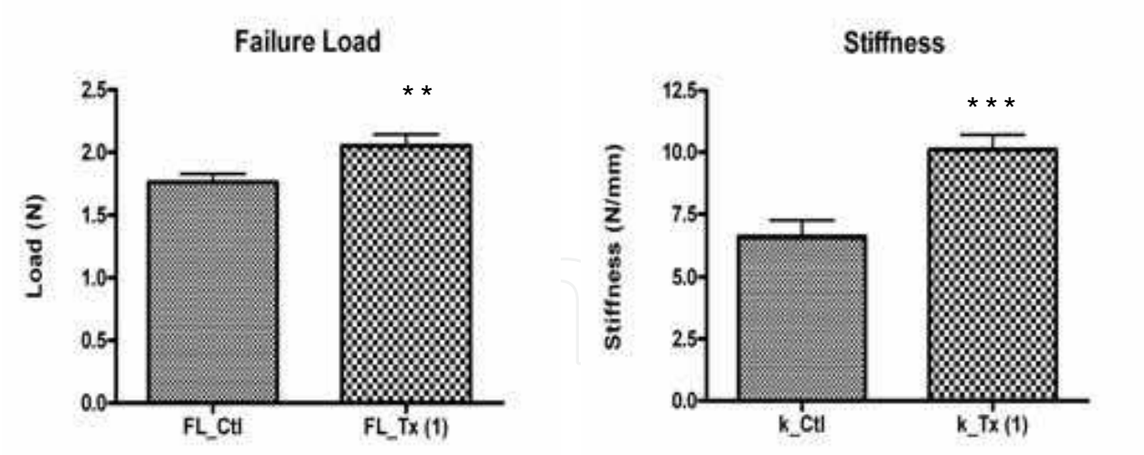


Fig. 18. Distraction in organ culture increased bone strength. Failure load (FL) and stiffness (k) were significantly increased in comparison to their no-load counterparts maintained in culture for 1 wk.

At this point, it has preliminarily been shown that organ culture systems may hold some potential as biomimetic models of bone cell mechanotransduction. Additional goals would be to expand the age of the organs beyond the neonatal stage. While the neonatal model has a higher chance of maintaining viability in culture given the increased porosity and decreased mineralization in comparison to adult bones, the neonatal system is somewhat limited in its usefulness. For instance, these models would not be appropriate for addressing issues related to osteoporosis. Where one could argue that osteoporosis is a disease indicative of increased porosity and bone destruction and would therefore likely have a reasonable life in culture, the control for an osteoporotic model would be an adult, normal bone. The adult bone would have an increased level of mineralization that would not be conducive to organ culture. Thus, it may prove helpful to investigate means to increase culture viability to enhance survival of mineralized, mature bones. Two obvious avenues of research would be to optimize culture conditions with topical additives and/or to develop active perfusion systems. Current efforts to develop the perfusion chambers are underway and chamber dimensions have been developed based upon laminar flow profile analysis, Figure 19. Solid modeling software and time dependent fluid flow analysis were utilized to develop a chamber that enabled fully developed laminar flow to reach the bone. Rapid prototyping techniques have been employed to fabricate the chambers and studies are ongoing to determine a flow profile that prolongs culture viability, without providing measurable stimulation to the bones.

Efforts are also underway to demonstrate that additional bone models are appropriate for organ culture studies. Given the need for models that can be used for craniofacial DO studies and the need to develop planar models of loading that more appropriately model clinical DO scenarios, studies examining the viability of mandibles in culture have also been initiated. Prior to applying load to mandibular models, it is necessary to characterize the development of the mandibles in organ culture. As shown in Figure 20, mandibles maintained in culture for 4 wk stain heavily for calcium content and display a resorption pattern consistent with tooth eruption. In addition, although bone is lost as a result of this possible eruption, the ash weight of mandibles at 4 wk of culture is significantly greater than that of controls taken at 24 hr, increasing 22% (data not shown). Current microCT studies

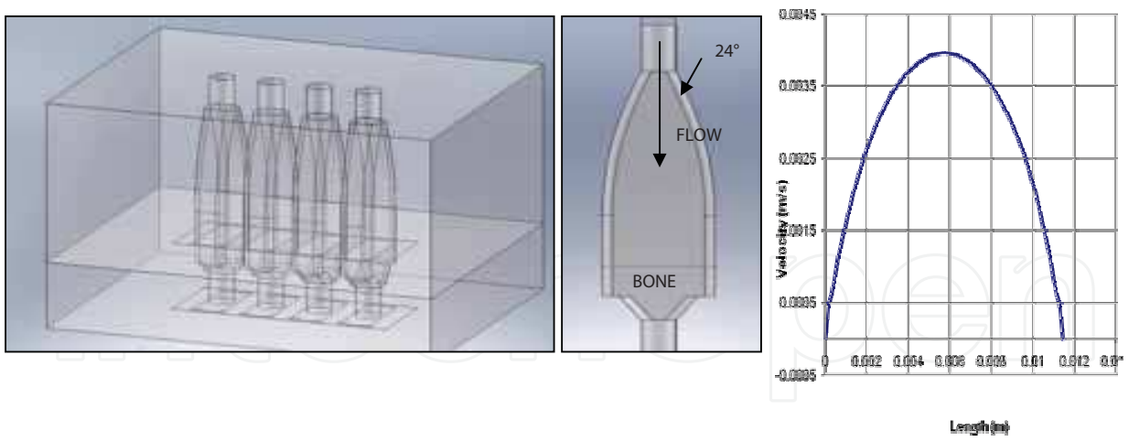


Fig. 19. 3D solid model of a perfusion chamber providing a laminar flow profile to organ culture in an effort to prolong culture viability and extend the biomimetic systems to include adult, mineralized bone models.

Fig. 20. (Left) Alizarin red staining at 4 wk in cultured demonstrated the presence of calcium and good bone quality and quantity. (Right) 10 micron H&E sections (decalcified) demonstrated the presence of healthy bone, tooth development and model viability (presence of cellular nuclei) at 4 wk of culture.

are underway to determine if tooth development in culture parallels that of age-matched controls to establish baseline culture properties prior to applying distraction. While only preliminary work has been initiated in this area, continued work will help to determine the validity of organ culture systems for biomimetic mechanotransduction modeling of craniofacial distraction osteogenesis. As this work progresses, new mechanical loading devices will need to be developed which will apply the appropriate vectors to the bone and will ultimately enable 2D and 3D distraction.

Bone, because of its unique ability to adapt to its environment will always be a target of biomimicry. Its innate modification to structural design to prevent failure is an extremely attractive property of bone that if emulated could have countless benefits to the public and private sectors. While biomimetics often cites bone as inspiration, our understanding of bone's ability to adapt to its environment is still in its nascency and biomimetic bone models

may help elucidate the response of bone to mechanical stimulation. Understanding this response would have significant benefit to the medical field and could aid in the improved design and fixation of implant systems, functional tissue engineered bone constructs, bioreactor development and the eradication of metabolic bone diseases such as osteoporosis. As such, the focus of this work has been to introduce the reader to the field of bone mechanotransduction research by focusing on the engineering systems and models currently available. Furthermore, the objective was to introduce the reader to the concept of an organ culture, or *ex vivo* mechanotransduction system that we believe may be more biomimetic than *in vitro* models and less complex than *in vivo* models. Laying the groundwork for biomimetic models in bone mechanotransduction research, we demonstrated that neonatal, whole bone organ culture models remain viable, continue to grow and are osteogenic in response to mechanical stimulation. Furthermore, we demonstrated in a variety of preliminary studies that these models may prove useful in mechanistic studies requiring culture additives and are capable of simulating clinical procedures, such as distraction osteogenesis. Groundwork has been laid to develop perfusion chambers to prolong culture viability and the characterization of additional bone models in culture is underway to assess their future usefulness in mechanotransduction research. However, a major question that remains to be answered regarding the organ culture systems is to determine whether or not the pathways and mechanisms by which the bone cells respond are altered in the organ culture system. That is, does the bone respond along the same mechanisms and pathways to stimulation in culture that it would if maintained in the body, or do compensatory mechanisms/pathways take over such that the very nature of the response is altered by the use of the organ culture? In engineering terms, this would be equivalent to the concern as to whether or not the measurand affects the measurement. While only time will tell if the organ culture model will prove to be acceptable for biomimetic mechanotransduction studies, the initial work is promising.

Appendix: Derivation of fluid shear stress generated with a parallel plate flow configuration:

Starting with the Navier-Stokes equation assuming steady, incompressible and fully developed flow

$$\rho g_x - \frac{\delta P}{\delta x} + \mu\left(\frac{\delta^2 u}{\delta x^2} + \frac{\delta^2 u}{\delta y^2} + \frac{\delta^2 u}{\delta z^2}\right) = \rho\frac{\delta u}{\delta t}$$

$$\frac{\delta P}{\delta x} = \frac{dP}{dx}$$

$$\left(\frac{\delta^2 u}{\delta x^2} + \frac{\delta^2 u}{\delta y^2} + \frac{\delta^2 u}{\delta z^2}\right) = \frac{d^2 u}{dy^2}$$

$$\frac{\delta u}{\delta t} = 0$$

The Navier-Stokes equation becomes

$$\mu\left(\frac{d^2u}{dy^2}\right)=\frac{dP}{dx}=C=-K$$

$$\left(\frac{d^2u}{dy^2}\right)=\frac{-K}{\mu}$$

Integrating twice

$$u(y)=\frac{-Ky^2}{2\mu}+C_1y+C_2$$

And given the no slip condition at the boundaries

$$u=0;@\ y=\pm\frac{h}{2}$$

$$@\ y=\frac{h}{2};\quad 0=\frac{-K}{2\mu}\left(\frac{h^2}{4}\right)+C_1\frac{h}{2}+C_2$$

$$0=-K\left(\frac{h^2}{8\mu}\right)+C_1\frac{h}{2}+C_2$$

And

$$u=0;@\ y=-\frac{h}{2}$$

$$@\ y=-\frac{h}{2};\quad 0=\frac{-K}{2\mu}\left(\frac{h^2}{4}\right)+C_1\left(-\frac{h}{2}\right)+C_2$$

$$0=-K\left(\frac{h^2}{8\mu}\right)-C_1\frac{h}{2}+C_2$$

Adding equations to solve for C_2

$$-K\left(\frac{h^2}{8\mu}\right)+C_1\frac{h}{2}+C_2+\left(-K\left(\frac{h^2}{8\mu}\right)-C_1\frac{h}{2}+C_2\right)$$

$$C_2=K\left(\frac{h^2}{8\mu}\right)$$

Substituting to solve for C_1

$$0=\frac{-K}{2\mu}\left(\frac{h^2}{4}\right)+C_1\left(\frac{h}{2}\right)+K\left(\frac{h^2}{8\mu}\right)$$

$$0=C_1\left(\frac{h}{2}\right);\quad C_1=0$$

The equation takes the form

$$u(y) = \frac{-Ky^2}{2\mu} + \frac{Kh^2}{8\mu}$$

$$u(y) = \frac{K}{8\mu}(h^2 - 4y^2)$$

The volume flow rate (Q) may be determined by integrating the velocity (u) over the flow chamber's cross-sectional area

$$Q = \int_{\frac{-h}{2}}^{\frac{h}{2}} \frac{K}{8\mu}(h^2 - 4y^2)\,(b\,dy)$$

$$Q = \frac{bK}{8\mu} \int_{\frac{-h}{2}}^{\frac{h}{2}} (h^2 - 4y^2)\,dy$$

$$Q = \frac{bK}{8\mu} \left(h^2 y - \frac{4}{3}y^3\right)\Big|_{\frac{-h}{2}}^{\frac{h}{2}}$$

$$Q = \frac{bK}{8\mu} \left[\frac{h^3}{2} - \frac{4h^3}{24} - \left[-\frac{h^3}{2} + \frac{4h^3}{24}\right]\right]$$

$$Q = \frac{bK}{8\mu} \left[\frac{2h^3}{2} - \frac{8h^3}{24}\right]$$

$$Q = \frac{bK}{8\mu} \left[\frac{2h^3}{3}\right]$$

$$Q = \frac{bh^3K}{12\mu}$$

Since wall shear stress is defined as

$$\tau_w = \mu \frac{du}{dy}\Big|_{y=\pm\frac{h}{2}}$$

$$\tau_w = \mu \frac{d}{dy}\left(\frac{K}{8\mu}(h^2 - 4y^2)\right)\Big|_{y=\pm\frac{h}{2}}$$

$$\tau_w = \frac{K}{8}\left(\frac{d}{dy}(h^2 - 4y^2)\right)\Big|_{y=\pm\frac{h}{2}}$$

$$\tau_w = \frac{K}{8}(0 - 2(4y))\Big|_{y=\pm\frac{h}{2}}$$

$$\tau_w = \frac{K}{8}(-8y)\Big|_{y=\pm\frac{h}{2}}$$

$$\tau_w = (-Ky)|_{y=\pm\frac{h}{2}}$$

$$\tau_w = -K\frac{h}{2}$$

Upon substituting back

$$Q = \frac{bh^3K}{12\mu} = \frac{Kh}{2}\left(\frac{bh^2}{6\mu}\right) = \tau_w\left(\frac{bh^2}{6\mu}\right)$$

$$\tau_w = \left(\frac{6\mu Q}{bh^2}\right)$$

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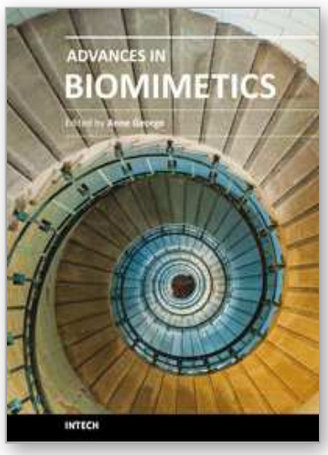

Advances in Biomimetics

Edited by Prof. Marko Cavrak

ISBN 978-953-307-191-6
Hard cover, 522 pages
Publisher InTech
Published online 26, April, 2011
Published in print edition April, 2011

The interaction between cells, tissues and biomaterial surfaces are the highlights of the book "Advances in Biomimetics". In this regard the effect of nanostructures and nanotopographies and their effect on the development of a new generation of biomaterials including advanced multifunctional scaffolds for tissue engineering are discussed. The 2 volumes contain articles that cover a wide spectrum of subject matter such as different aspects of the development of scaffolds and coatings with enhanced performance and bioactivity, including investigations of material surface-cell interactions.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Marnie M Saunders (2011). Biomimetics in Bone Cell Mechanotransduction: Understanding Bone's Response to Mechanical Loading, Advances in Biomimetics, Prof. Marko Cavrak (Ed.), ISBN: 978-953-307-191-6, InTech, Available from: http://www.intechopen.com/books/advances-in-biomimetics/biomimetics-in-bone-cell-mechanotransduction-understanding-bone-s-response-to-mechanical-loading

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