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Theoretical Study on Conduction of Electric Charges across the Trabecular Components of Bone When Intact and During Healing Of a Bone Fracture.

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ABSTRACT

This paper discusses the function of bone cells, the properties of bone matrix and the electric properties of bone in general. The conduction characteristics of the bone that changes during the fracture, formation of unmineralized bone matrix and calcified matrix are also discussed theoretically.

Keywords: electric charges, trabecular, bone, healing, fractures

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INTRODUCTION

The frequently asked question, when a patient visits a doctor during a fracture treatment is whether his fracture has united. In the recent past the common method that was followed was radiography. There were problems like repeated exposure to patient and the radiographer. There were also problems of inter-observer variations. Recently direct electric current is applied diagnostically for the first time in bone fracture patients treated with Ilizarov. In these studies it was found that stabilization of electric output current indicated a healed -bone fracture and application of direct electric current is safe [1-5]. We have also written on the perspectives on the studies using electric parameters to study fracture healing in tibia [6]. The empirical nature of the studies and the inability to completely isolate the bone conduction is accepted to be impossible [6].

In this paper we wanted to study theoretically the basic molecular structure of bone, the factors that could have affected the electric charge conduction during our experimental studies in 32 tibial bone fracture patients [1-5].

The first part of this paper generally reviews the bone cells, matrix, components of bone, marrow as conducting part, the anisotropy of bone, energy absorption and distribution and the structural difference between the cancellous and cortical bone and other factors affecting conductivity. The second part of this paper theoretically analyses the path of conduction with regard to the lamellar structure, in normal, in fracture with hematoma, in fracture with un mineralized callus, and in ossified callus in fracture.

Bone cells

Basic histology of bone is not the aim of this paper but there are certain facts that need to be reiterated. Bone cells are entrenched within the cortical bone. They derive the nutrients only through canaliculi from the central marrow cavity in cortical bone. The shape of the bone forming cells depends on the stage of the bone formation. When a bone is mature, there is no active process of remodeling. During this time the osteo-progenitor cells lie flat on the surface of the bone. In contrast in active bone turnover stage, the cells become large and palisadic [7].

Matrix formation

Thus only during the bone turnover the cells become bigger. The osteoblast secretes collagen and un mineralized bone matrix. The osteoblast deposit the unmineralized bone matrix, later are engulfed by the unmineralized bone matrix itself and become osteocytes. These osteocytes release calcium from the bone into blood. One should note that because of the size of both the osteoblast and the other bone lysing cell called the osteoclast, does not allow them to function inside the cortex. So they can function only on the surface. The calcification of this un mineralized bone matrix completes the bone formation. As already pointed out the larger cells, the osteoclasts (150microns diameter) lyses the calcium by secreting acid and lyses the collagen by enzymes [7].

Components of bone

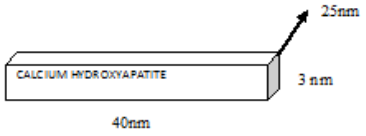

Figure 1. Structure of Calcium Hydroxyapatite

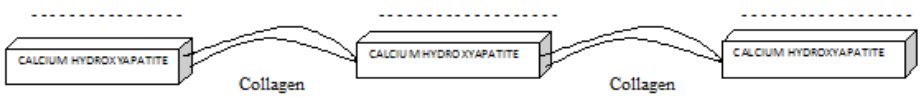

Figure 2. Calcium Hydroxyapatite Embedded in Collagen and the charged nature of the Calcium Hydroxyapatite

65 % of bone is composed of mineral (inorganic part)comprising of Calcium, Phosphorus, Magnesium, Sodium and Potassium. In this portion, Calcium and phosphorus form crystalline $Ca_{10}(PO_4)_6(OH)_2$ form a rod like structure as shown in figure 2.

60 percent of bone matrix water is free and remaining 40 percent is bound. Surface ions of the calcium hydroxyl apatite are hydrated. This helps in ionic exchange between crystals and body fluids. [7] The embedded structure of the Calcium Hydroxyapatite in collagen background is shown in figure 2.

Marrow as the main conducting part

The bone marrow occupies the non mineralized central portion of the cortical bone. The blood in this marrow space and fluid in the canaliculi of the Haversian system are the major source of conduction of charges [7].

Anisotrophy as an adaptation

Before considering any conduction, one should visualize bone as an anisotrophic structure. " if Young's modulus is referred, its dependence between the density and Young's modulus is reported in different multiples e.g. in cubic or squared for multiple sites analysed together or for a single site it is linear when the sample of bone is loaded along the lines of the trabecula '' [8]. Such a difference is due to the workers neglecting the anisotrophic properties of bone [8]**.** Since bone is usually exposed to various external forces and stress, such a property of different elastic modulus in different directions must be considered as an adaptive response to functional loading. It is well explained in a vertebra, where it can take 3.4 times more load vertically than transversely [8]. By Wolff's law "under the direction of functional pressure the given form of bone, changes by placing or displacing its elements". Thus, anisotrophy should be considered as a type of adaptive response to functional loading. Trabecular bone shows orthotrophic symmetry.Bulk electrical properties of tissues of the body determine the path of flow of current through the body. This is considered as vital in the analysis of interaction between the electromagnetic waves and the human body [9].

Energy absorbed and distributed

Another important knowledge is finding how much energy is absorbed, in what rate, how it is distributed in the human body? [9]. This is mainly important in theoretical application of ECG, muscle contraction etc.

Connectivity is an important factor; there is a difference between the cortical bone where there is node to node type of connection than node and branch type connection in cancellous bone. i.e. there will be more than one path of conduction in cancellous bone between two nodes. Conductivity is the readiness of charged particles to move through the material under the influence of an electric field. In biological materials changes are involved with 1.polar molecules, 2.electrical double layer near membrane or solvated macromolecules. When an external electric field is applied the system is disturbed with each polar and polarizable particle responding by a change in their own way. When this external electric field is removed, the above disturbed particles relax to attain a new equilibrium with relaxation time called tau τ [9]. This is for one particular process excitation and relaxation of one molecule. There are multiple processes that are happening in the human tissue. When there is a decrease in permittivity or increase in frequency the conductivity increases. For a given voltage the total energy of the electric field is constant. This energy can be stored (real value) by the material ϵ' or dissipated (imaginary value) by it ϵ'' Also a fall in permittivity causes increase in frequency. This is so because various electric charges cannot follow changes in applied electric filed. When the frequency of the current is increased, there are four levels of falls α, β, γ and in addition is also described in Giga Hertz frequencies, the δ. These are in cell suspensions , however in live tissues there might be overlaps of dispersion with almost a continuous fall with no plateau, with increasing frequencies.There are many problems when an electrical system is used, this is because the electrode resistance properties or impedance is finite. This leads to complex electrothermic reaction at the electrode - tissue interface causing polarization phenomenon. When a metal electrode is placed in a biological tissue and connected to a generator, at the electrode tissue interface, faradic current results. Around this a capacitative (non faradic) current develops from the previous double layer. This second layer acts like a capacitance. The current across the interface charges this double layer and results in polarization [10]. Figure 3

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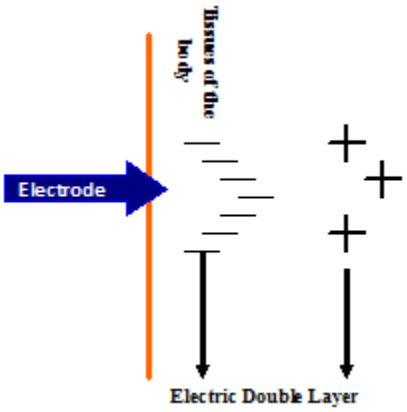

Figure 3: Electrode inside a biological system

Using Ohm's law is possible, when recorded signals are determined mainly by 1.Input stimulation,2. Electrical parameter of the tested material 3.Geometry of the electric system. This sort of linear assumption is possible if electric field is uniform and is undisturbed by other objects with cells. The errors that are introduced by the measurement cell are evaluated with phantom material as a function of frequency and geometry of the cell [10]. Increasing the surface area of the electrode decreases the cut off frequency thus if the electrode is made up of larger surface area it will also affect conduction [11]. Silver chloride electrodes are considered to be ideal but exposure to light can change their potential and the coating can be eroded by abrasion. The roughness of the stainless steel surface is essential for conduction but it gets smoothened with time. The frequency dependence of electric properties of bone are comparable with ligaments i.e tissue of less cell content. The comparatively low water content of these tissues may be the reason for the differences in permittivity values between tissues with low and high cell content [11]. Main contribution for the relative permittivity is from polarization of ions in the surface of the charges on the charged surface of bone. Numerous interfaces are there between lamellae fibrils capillaries will affect the conductive properties. The electrical properties of compact bone have been studied in dry bone and in wet bone. Wet bone resembles in-vivo conditions. Skinner etal has studied healing process during corticotomy and transport by Ilizarov followed by impedance spectroscopy [11].

The difference in porosity between the compact and cancellous bone.

Structural difference in porosity between the compact and cancellous bones exist (Figure 4a and 4b) but studies show the properties studied in dry state between these two types bones are very similar. These show that the electrical properties of calcific matrix in dried specimen of both compact and cancellous bones are very similar. But in a fluid saturated matrix things are different from the dry network.

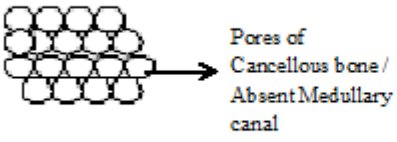

Figure 4a. Cancellous bone

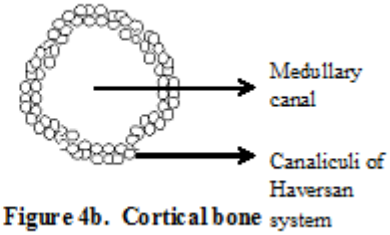

Figure 4b. Cortical bone

Thus decalcified matrix and the immersion fluid form the important interfaces that will decide the electrical properties of the bone. These are studied separately and various values are presented for the relative conductivity and permittivity is in different ranges and not in specific values. This is mainly due to the huge difference in the water content of the tissues. Trabecular bone was reported to be transversely isotrophic .Interestingly in osteoporotic persons these transverse trabeculae are selectively resorbed [11].

Other factors affecting conductivity

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In addition to the above factors, there are other factors that will affect the conductivity of bone. These are 1.the moisture content, temperature and immersion fluid and age and disease state of the individual. First and foremost is the moisture content. This is due to the rotational ability of water molecules. An increase in conductivity proportional to the moisture content .At 98 percent relative humidity, the large pores are empty and only small pores are filled with fluid. When tissue dries up, for example after a time of five minutes, there is an increase in resistance by 92 percent. In certain experiments there was an erroneous estimation from surface conductivity changes. 2. Next, an increase in the temperatures increases the conductivity. 3. The properties of the fluid previously told to fill the pores of the bone affect the conductivity of the bone. A change by 2 in the pH value causes a change by 70 % in the conductivity. Chemical changes affecting the conductivity of bone with ageing, are only assumed. Experimentally in rat bone dielectric properties are shown to change with changes in water content and organic tissue composition. Also quantitative differences between the species in animals are not estimated [11].

Part 2 a Theoretical analysis of the path of conduction across the trabecular structure

It should be highlighted that we are analyzing the conductive property of bone and not measuring the piezoelectric effect of the bone. According to Telega et al, the electromagnetic effect observed in the wet bone is not piezoelectric effect .The piezo electric moduli evaluated in the uniaxial compression by Fukada and Yasuda (1957), Liboff and Shamos (1971), combined with an expression derived by Williams and Breger (1974, 1975) for the voltage expected across a homogenous piezo- electric beam under cantilever bending did not describe the response of biological tissues, neither quantitatively or qualitatively. The results of the experiments on wet bone show in contrast to dry bone, the piezo electric effect in wet bone is insufficient and irrelevant. When bone, tendon and cartilage are subjected to mechanical forces under physiological conditions, piezo electric signals are not usually measured as induced polarization is rapidly neutralized by ions in the bulk fluid [12].

One can follow this paper easily if the one reads our earlier paper [6] and sees the figure 1 in that article. A simplified version of the same with regard to the local bone histology is serially given as fracture healed. a visualization of charge conduction across calcium hydroxy apatite when bone is intact is shown in figure 5.

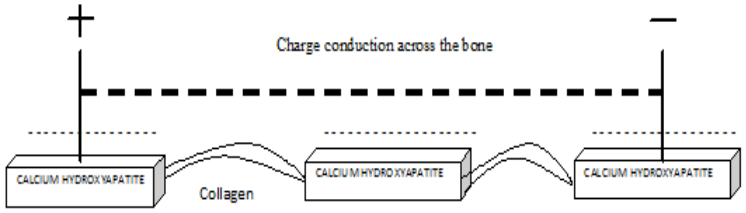

Figure 5 : Charge conduction across intact bone

Apart from crystalline calcium hydroxy apatite, there is also an amorphous form of calcium carbonate. Thus conduction from the first stage of blood in fracture hematoma, in un mineralized collagen and then in calcified fracture tissue results. The conduction property of each one is explained in stages.

Conduction during the stage of fracture hematoma

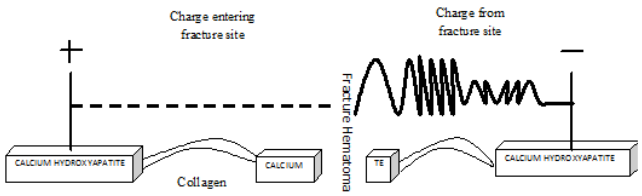

Figure 6 : Conduction during the stage of fracture hematoma

Figure 6 : Conduction during the stage of fracture hematoma

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One can imagine charge conduction from one marrow and lamella to fracture hematoma and to other marrow and lamella. (Figure 6) The marrow is not shown in the figure and only the cortex part is shown. This is possible if one assumes the other tissues are not irregular and maintain same level of conductivity throughout. A fracture occurs due to spending of an external energy after the limit of the bone's elasticity is exceeded. Blood from the medullary vessels flow into the bone fracture space. This blood usually clots if there is no clotting pathology. The serum with cells separated conducts more. The red blood cells are poor conductor of electricity. This property is used to find the relative share of these cells in blood. i.e. in anemia, the blood conducts more than normal blood [13]. This is confirmed *in vitro* with a range of indicators and canine blood with a tetra polar conductivity cell) [14]. The electrical conductivity of blood depends on its flow velocity. Thus the conductivity also changes when blood is static [15]. Hence when the cells are removed into a separate mass of clot, the fluid in serum conducts better. Thus for at least the first few weeks, till cells come inside the fracture hematoma, there is an increased conduction.

Conduction during the stage of an un mineralized matrix

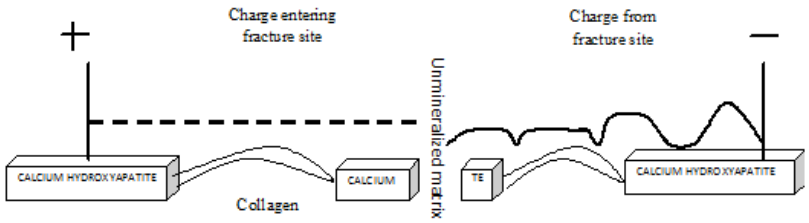

Figure 7. Conduction during the stage of an unmineralized matrix

With migration of the osteoblasts into the fracture hematoma, the osteoblasts become bigger secreting, unmineralized matrix. With slow replacement of the previously highly conducting fracture hematoma, with another material (unmineralized matrix) of different conducting properties, the conducting properties also changes to less spiky curves (Figure 7).

Conduction during the stage of a mineralized matrix in fracture site.

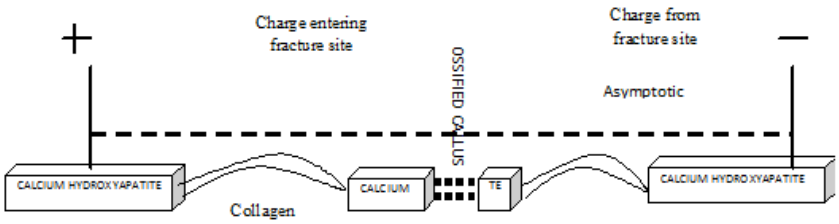

Figure 8. Conduction during the stage of a mineralized matrix in fracture site

With calcification of the already secreted, unmineralized matrix, there is slow conversion of the conducting unmineralized matrix to a mature callus of different conducting properties, i.e near the original conducting property of an intact bone which is a poor conductor. Thus the current output falls and becomes asymptotic (Figure 8).

Why the direction of the study is useful?

Given the theoretical deliberation of how the electric charges are conducted in intact and fractured bone , one should be surprised at its empiricity.To explain the importance of study and theoretical understanding in this direction the example of an ECG is cited. D.J. Rowlands says *'In excess of 100 million 12 lead ECGs are recorded annually world-wide , a fact that would surely would have astonished Waller (*Augustus Désiré Waller 1856-1922*) who made the first electrographic recording of the human heart in 1887 and*

*expressed the view that it was unlikely that such recording would be of much use in clinical practice. This view was however not shared by Einthoven (*1860-1927 who was awarded the Nobel prize in 1924 for discovering ECG*). Electrocardiography developed empirically and its basics diagnostic criteria remain empirical.'* One needs to accept this. With the main challenge of inability to completely insulate the electrodes and also the bone also, one can only say there is an empirical collective conduction measurement across the fractured limb as a whole [16]. Coming to the problems in assessing bone deposition in fracture callus I quote from Gerstenfeld LC et al [11] 'the basic structural mechanism used to remodel the callus is inverted from those of transverse bone growth. Unlike long one expansion where there is a balance between periosteal appositional growth and resorption at the endosteal surface, remodeling of fracture callus uses different and unique spatial mechanisms to model and by some mechanism must remodel from the outer surface inward, balancing the external removal with addition of new bone on internal surfaces. This aspect of fracture healing is structurally complicated and the ability to reconstruct a simple model to explain how this takes place is very challenging'. As the results show' more bone forms distal to the fracture site in femoral healing and proximal to the fracture in tibial healing. Further investigations will be required to better understand the complex interplay of each of these processes" [17].

Bovine and human bone

Experimentally there is clear difference between the bovine and human bone with regard to their density. When parallel plate capacitance method and co axial probe methods are used the measurements are different .Most studies find, the interstitial fluid as a main conduction determinant of the bone. Here the DC conduction is dominant in bone attributed to the structural arrangement of the trabeculae. The mineralized bone matrix contains insufficient amount of cells to significantly increase the amount of current paths.[18] This might be the reason of reduced conduction after mineralization. Also the marrow fat is a poor conductor of electric charge [18]. Dry bones sometimes show high conduction than predicted theoretically. This means the conduction can also be mainly affected by bound water. However in experiments with AC, in increasing frequencies, polarization in collagen and in bone marrow causes less conduction .In bone the protein collagen is surrounded by water molecules (bound water) which form hydrogen bonds. The rotation of this bound water molecules will determine the conduction and permittivity properties, especially the delta at GigaHertz. [18] The presence of large amount of bound water in the trabecular tissue also proves the DC conductivity part in the overall dielectric response of the tissue [18]. As already mentioned the less dense structure and more liquid caused more conduction in human bone than bovine bone. This water present on the surface of the bone and within the contents at low frequency current causes higher values of peak dissipation factors in the human bone [18]. According to the site also (e.g. metaphysis or diaphysis) ,the electrical properties vary. These may be due to differences in the amount and orientation of the collagen fibres. But such a difference is more in bovine than human bone [18].

Freezing of the harvested bone and its effects on the conductivity

In experiments freezing and thawing is done before samples are subjected to electric current. The significant changes in the conductivity occur within a few hours of death. In studies with very short period of freezing and thawing the changes are not rapid. Thus prolonged freezing hypothetically destroys the cell membrane and causes problems in electrical double layer formation between the proteins and lipids causing reduced conductivity. [18] The difference in the parallel plate probes and the open ended probes may be due to the different orientation of trabeculae in different directions.In live tissue, the high water and fat content with a low BMD causes a high DC conductivity masking the AC conductivity at low frequencies. Healthy bone have low conductivity, the conductivity mainly due to water in the interstitial tissues.Also interstitial bone marrow in cancellous bone also affects conductivity [18].

SUMMARY

The paper explains the theory of conduction of the electric charge across an intact and fractured bone with respect to its basic molecular structure of calcium hydroxyl apatite crystals linked with collagen. It also follows the stages of changes in conduction as fracture site blood becomes un mineralized matrix and later calcified bone. Both the structural arrangement of the trabeculae tissue and large amount of bound water makes DC conductivity dominate the overall dielectric response of the bone tissue. Further studies in this line are essential in human volunteers preferably noninvasively.

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