A comparison of the microarchitecture of lower limb long bones between some animal models and humans: a review

V.J. Cvetkovic[1], S.J. Najman[2], J.S. Rajkovic[1], A.Lj. Zabar[1], P.J. Vasiljevic[1], Lj.B. Djordjevic[1], M.D. Trajanovic[3]

[1]Faculty of Sciences and Mathematics, University of Nis, Nis, Serbia
[2]Faculty of Medicine, University of Nis, Nis, Serbia
[3]Faculty of Mechanical Engineering, University of Nis, Nis, Serbia

ABSTRACT: Animal models are unavoidable and indispensable research tools in the fields of bone tissue engineering and experimental orthopaedics. The fact that there is not ideal animal model as well as the differences in the bone microarchitecture and physiology between animals and humans are complicate factors and make model implementation difficult. Therefore, the tendency should be directed towards extrapolation of the results from one animal model to another or from animal model to humans. So far, this is the first paper which provides an overview on the microarchitecture of lower limb long bones and discusses data related to osteon diameter, osteon canal diameter and their orientation, as well as intracortical canals and trabecular tissue microarchitecture in commonly used animal models compared to humans depending on age, gender and anatomical location of the bone. Understanding the differences between animal model and human bone microarchitecture should enable a more accurate extrapolation of experimental results from one animal model to another or from animal models to humans in the fields of bone tissue engineering and experimental orthopaedics. Also, this should be helpful in making decisions on which animal models are the most suitable for particular preclinical testing.

Keywords: bone microarchitecture; lower limb; osteon; intracortical canals; trabeculae; animal models

Contents

1. Introduction

Bone is a hierarchically organised tissue which adapts and changes under the influence of the environment and according to internal body factors. Bone structure is very variable among species but also in the body of one specimen depending on bone function and specific factors like age, gender, general condition and lifestyle (Jowsey 1966; Mavropoulos et al. 2007; Podshivalov et al. 2008). For example, human vertebrae cancellous bone is mostly made of rod-like trabeculae, while the femoral head mainly consists of plate-like trabeculae (Podshivalov et al. 2008). Bone structure can also

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vary in different anatomical locations of the same bone because of different functional requirements (Wolff 1892; Hert et al. 1994). This phenomenon is partly defined by Wolff's Law, which states: "Every change in the form and function of bone or of its function alone is followed by certain definite changes in the bone internal architecture and equally definite alteration in its external conformation, in accordance with mathematical laws" (Wolff 1892). Understanding bone microarchitecture is essential for understanding the functioning of healthy bone and also of the bone in the processes of regeneration.

First steps towards the description of bone microarchitecture in animals and humans were made already in the 19th century (Jowsey 1964, 1966; Wolff 1892). Recently, however, great progress has been made in this field (Hert et al. 1994; Bagi et al. 1997; Ardizzoni 2001; Sugawara et al. 2005; Tromp et al. 2006; Cooper et al. 2007; Mavropoulos et al. 2007; Martiniakova et al. 2008; Britz et al. 2009; Pazzaglia et al. 2010; Schneider et al. 2010). Nevertheless, only a few papers comparing bone microarchitecture between animal models and humans have been published so far (Jowsey 1966; Bagi et al. 1997; Pearce et al. 2007). Furthermore, there aren't any papers which integrate data on bone microarchitecture of lower limbs long bones in frequently used animal models, i.e., rabbits and rats.

In vivo research in experimental and veterinary orthopaedics and bone tissue engineering is indispensable which implies the usage of different animal models. As all animal models have advantages and disadvantages for their use in preclinical testing they must be considered only as an approximation of humans. Pearce et al. (2007) concluded that dogs have the most similar bone structure to humans while rabbits have the least similar bone structure to humans. However, there are many ethical concerns related to the use of dogs as well as primates which also have the most similar bone structure to humans. Considering ethical issues, handling, housing, cost of cultivation and other factors it can be concluded that rabbits and rats are still the most suitable for experimental orthopaedics and bone tissue engineering research.

According to our experience with rats and rabbits as animal models in bone regeneration studies, there is a need for a systematic overview on bone microarchitecture, especially in the femur and tibia which are some of the most common bone models in bone defect studies (Najman et al. 2004; Ajdukovic et al. 2005; Kalicanin et al. 2007; Janicijevic et al. 2008; Vasiljevic et al. 2009; Vukelic et al. 2011). The goal of this review is to describe the differences in the microarchitecture of bone tissue primarily in the most frequently used models (i.e., rabbits and rats) and human lower limb long bones depending on gender, age and different lifestyles. As animal models represent the gold standard in preclinical studies, the tendency should be towards an extrapolation of the results from one animal model to another or from animal models to humans. Extrapolation of results is very important because it reduces the number of experiments on similar topics and contributes to the quality of interpretation of results. However, differences in bone microarchitecture and physiology between animals and humans complicate and make it difficult. Nevertheless, it is not impossible.

2. Long bone structure

Briefly, long bones of lower limbs consist of a diaphysis or shaft and epiphysis or extended ends whose surfaces are covered with articular cartilage that forms articulating surfaces. Between the diaphysis and epiphysis there are short, wide segments – metaphyses that represent the transition between the epiphysis and diaphysis (Ross et al. 1989; Buckwalter et al. 1996). The medullary cavity, which is filled with bone marrow, is located in the diaphysis and surrounded by a wall of cortical bone. Cancellous bone makes a very thin layer on the inner wall of the diaphysis of long bones which improves the mechanical properties of cortical bone (Buckwalter et al. 1996). However, metaphyseal and epiphyseal parts are richer in cancellous bone tissue consisting of numerous trabeculae which are connected to build an irregular network with a system of canals and cavities filled with bone marrow. On a cross section of long bones there are four different bone types: periosteum, cortical bone, endosteum and cancellous bone (Ross et al. 1989).

According to Podshivalov et al. (2008) complex multilevel structure of bone tissue consists of five structural levels: Macro-structural (mm-μm) – trabecular and cortical bone tissue diameter; Microstructural (10–500 μm) – osteon and trabeculae; Sub-microstructural (1–10 μm) – lamelae and individual trabeculae; Nano-structural (100 nm–1 μm) – collagen and minerals deposited in the matrix and Sub-nanostructural (less than 100 nm) – molecular

components of collagen and protein molecules. In this paper, we will focus on the macro- and microstructural level of bone structure.

2.1. Microarchitecture of the cortical bone

Bone tissue is divided into cortical and cancellous bone tissue. Proportions of cortical and cancellous tissues in bones vary between different mammalian species as well as in different bones in the body of the same specimen. The porosity of the cortical tissue is about 10% (Buckwalter et al. 1996). The basic structural unit in the cortical bone of adult mammals is osteon or the Haversian system (Hert et al. 1994; Schneider et al. 2010). Osteon consists of concentric lamellae of bone matrix arranged around Haversian canals (Figure 1). Further, osteons are separated from each other by interstitial lamellae and on the inner and outer surface of the cortical bone there are the inner and outer circumferential lamellae. Additionally, it was found that osteons in humans consist of two different types of lamellae: compact lamellae that are rich in collagen and without osteocytes (acellular lamellae), and the loose lamellae that are poor in collagen but rich in osteocytes (cellular lamellae) (Marotti and Muglia 1988; Marotti 1996). Longitudinal Haversian canals are linked with transverse Volkmann canals and make the system of canals in bone tissue. Nerves and blood vessels that supply the osteocytes and mature bone cells with nutrients and oxygen pass through this canal system (Levick 2004). Volkmann canals are clearly different from Haversian because they are not surrounded by concentric cacanals are clearly different from Haversian because they are not surrounded by concentric lamellae like Haversian canals. The cement line represents the outer osteon boundary; it contains less collagen than the surrounding tissue and is rich in sulphur, proteoglycans and glycoproteins (Martin et al. 1998).

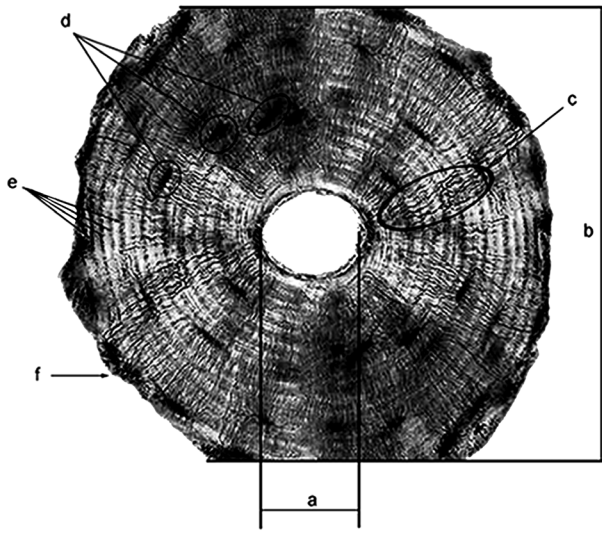

Figure 1. Osteon structure scheme. a = Haversian canal diameter; b = osteon diameter; c = lacuno-canalicular network; d = lacunae; e = concentric lamellae; f = cement line

Generally, it is accepted that osteon is formed by the remodelling effect of BMU (basic multicellular unit) which consist of osteoblasts and osteoclasts, due to the growth, repair and bone remodelling (Parfitt et al. 1994; Marks Hermey 1996; Martin et al. 1998; Mohsin et al. 2002). Osteon development in cortical bone occurs simultaneously with the progression of the vascular network so that the structure of the vascular network almost fits the intracortical canal system (Lee et al. 1965; Harris et al. 1968; Pazzaglia et al. 2007). In humans, as in other mammalian species, osteon structure and geometry depend on the age, sex, height and weight of individuals, genetic differences, differences in lifestyle, and the direction of mechanical loads during locomotion (Britz et al. 2009).

2.1.1. Osteon and Haversian canal diameter

Table 1 presents data on the osteon and Haversian canal diameters in the cortex of the femur in humans, rhesus monkeys, rats and rabbits depending on sex, age and anatomical position. Osteon diameters in the cortical bone of the femur in humans from two different studies Jowsey (1966) and Britz et al. (2009) are match each other very closely (Table 1), but in the group of younger people it is larger than in the group of elderly people (Jowsey 1966). This could be explained by a reduced activity of cells involved in remodelling processes with age (Martin et al. 1980). In addition, osteon diameter decreases with age, not only in humans but also in monkeys and rats (Currey 1964; Singh and Gunberg 1971; Martin et al. 1980; Havill 2003). On the other hand, the diameter of the Haversian canal in younger people is smaller than in older individuals and tends to increase with age. It was found that the average Haversian canal diameter in younger people (under 50) is 156 μm and in the elderly (over 50 years) it is 212 μm (Jowsey 1966). A similar pattern of Haversian canal diameter increase is confirmed by recent research (Cooper et al. 2007). However, in the compact bone of ribs the diameter

Table 1. Osteon and Haversian canal diameter in the femoral cortex depending on species, gender, age and anatomical location

Species	Gender	Age	Anatomical location	Osteon diameter (μm)	Haversian canal diameter (μm)	References
Homo sapiens	♀/♂	20–29 years	shaft	252 ± 24	151 ± 25	Jowsey 1966
Homo sapiens	♀/♂	50–59 years	shaft	235 ± 13	195 ± 11	Jowsey 1966
Homo sapiens	♀/♂	58 years	shaft	220	_	Britz et al. 2009
Homo sapiens	♀/♂	54.6 ± 21.2 years	shaft	_	117.2 ± 89.6	Cooper et al. 2007
Homo sapiens	♀	53.4 ± 23.6 years	shaft	_	150.4 ± 118.9	Cooper et al. 2007
Homo sapiens	♂	55.3 ± 19.9 years	shaft	_	99.3 ± 63.4	Cooper et al. 2007
Rhesus sp.	♀/♂	_	shaft	216 ± 52	167 ± 46	Jowsey 1966
Oryctolagus cuniculus	♂	2,5 months	shaft	199.83 ± 49.64	26.22 ± 5.73	Martiniakova et al. 2008
Oryctolagus cuniculus	♀	2,5 months	shaft	231.38 ± 44.82	27.00 ± 5.94	Martiniakova et al. 2008
Oryctolagus cuniculus	♂	8 months	shaft	_	30.56 ± 9.91	Pazzaglia et al. 2010

of the Haversian canal remains approximately the same size throughout lifespan, an average of 165 μm, and does not vary significantly as confirmed by other studies, too (Takahashi et al. 1965; Jowsey 1966). This example could be a proof that there is no identical pattern of Haversian canal diameter increasing with age in different bones of the same organism. These differences exist probably because changes in the bone microarchitecture during lifespan occur under the synergistic influence of different factors but not under the influence of any single one.

Nevertheless, the trend of osteon diameter decreasing and Haversian canals increasing with age in lower limb long bones, or osteon wall thinning, can be clearly seen from the data shown in Table 1. This clearly suggests that the osteon wall is thicker in young people, which is probably one of the reasons for the greater durability and stiffness in their bones. Besides this, it is interesting that in humans it is observed that the osteon becomes more regular (rounder) with age. In younger people osteon shape at the cross section is rather irregular (Currey 1964; Britz et al. 2009). Also, it was found that lighter people have a much larger diameter of cortical canals than heavier individuals (Cooper et al. 2007). It follows that heavier people have thicker osteon walls leading to proportionally stronger and more durable bones which in turn can respond to higher loads (Britz et al. 2009).

Inside the osteon, it was found that the average thickness of the loose lamellae from the cement line direction to the Haversian canal is 5.5 μm, 4.5 μm and 3 μm, respectively, while the thickness of the compact lamellae does not vary significantly, averaging about 1.5 μm (Ardizzoni 2001). Considering that the loose and solid lamellae are alternately shifting in the osteon wall, the space between solid lamellae decreases from cement lines towards the Haversian canal, and they get closer to each other in this direction. It is believed that the biological significance of such a structure is the secondary osteon strengthening from the cement line towards the Haversian canal (Ardizzoni 2001).

The diameter of the Haversian canal in rabbit femur in two different studies by Martiniakova et al. (2008) and Pazzaglia et al. (2010) are closely matched despite the differences in age of the tested animals (Table 1). The primate genus Rhesus has osteon and Haversian canal diameters most similar to humans (Table 1), probably because of genetic similarity and similar demands during locomotion. It is clear from Table 1 that osteon wall thickness is greater in rabbits than in humans and rhesus monkeys which can be associated with a different load on the femur in these species determined by locomotion and lifestyle. In addition, in another study it was found that juvenile chimps have a larger diameter of the osteon then the macaque monkeys while the size of the Haversian canal is approximately the same in the two species (Mulhern and Ubelaker 2009). These data suggest that the osteon wall is thicker in chimpanzees because of the influence of high mechanical forces which is the result of higher body weight in chimps and differences in locomotion and lifestyle (Schaffler and Burr 1984; Mulhern and Ubelaker 2009). However, chimpanzees have significantly smaller osteon compared with man (Mulhern and Ubelaker 2009). The bio-

logical significance of smaller osteon formation lies in a response to a specific lifestyle (jumping, using all four limbs during locomotion etc.) which determined that weight-bearing bones require increasing resistance to micro and macro fractures (Frost 1990; Mohsin et al. 2006, van Oers et al. 2008).

In rabbit femoral cortical bone, it was shown that Haversian canals are not continuous straight tunnels but successive shifts of straight and deviant segments that occur at branching points (Pazzaglia et al. 2009). Branching angles typically range from 10° to 30° and 150° to 170° and there is also a branching angle of 90°, while nodes at branching points are usually composed of three, four or five branches with the largest proportion of nodes with three branches (Pazzaglia et al. 2009).

There are a lot of data about differences in the structure of bone tissue between the sexes, both in humans and experimental mammals (Thompson 1981; Burr et al. 1990; Scheuer 2002; Cooper et al. 2007; Martiniakova et al. 2008; Britz et al. 2009). Data about the difference in osteon and Haversian canal size between men and women vary greatly. Some suggest that the diameter of the osteon and diameter of Haversian canal in women is greater than in men, while other studies suggest the opposite. Less frequently there are claims that there are no significant differences between them (Thompson 1981; Burr et al. 1990; Pfeiffer 1998; Cooper et al. 2007; Britz et al. 2009). In the primate species *Macaca mulatta* it was found that osteon densities are higher in females than in males (Havill 2003). Also, in rabbits the diameter of the osteon is significantly different between the sexes; it is larger in females than in males, which the authors explain with the different lengths of the femur between sexes. However, this is not the case with a Haversian canal diameter which is approximately the same size in different sexes without significant differences (Martiniakova et al. 2008). In *Bos aurus*, *Ovis aries*, *Sus scrofa domestica*, and *Equus caballus*, it was concluded that there were no significant differences between the sexes in bone microarchitecture (Urbanova and Novotny 2005).

2.1.2. Osteon orientation

In the cortex of diaphysis of the long bones in man (femur, tibia, humerus, radius) osteons are predominantly orientated longitudinally but they are not absolutely parallel to the longitudinal axis of the bone. The most prevalent opinion is that the osteons are oriented parallel to the direction of the loading force (Fiala and Hert 1993; Hert et al. 1994; Baca et al. 2007). Most of the osteons are oriented in a direction skewed from the longitudinal axis and the inclination angle varies, depending on the type of bone, from 0° to 15°. For example the mean inclination angle in the human femur is 8.1° (Hert et al. 1994). Further, osteons are set to form two helical systems which are placed opposite one another at opposite sides of the diaphysis. Also, one helical system is larger than the other due to specific effects of torque moments and due to maximum principal strain in everyday lifestyle (Lanyon and Bourn 1979; Hert et al. 1994). In addition, a similar osteon arrangement was found in the sheep radius where osteons are slightly twisted orientated from the bone axis and in the dog femur where osteons are orientated in a helical way around the bone shaft in a clockwise direction in the right femur, and in the same pattern but counter clockwise in the left femur (Cohen and Harris 1958; Mohsin et al. 2002).

2.1.3. Haversian canal density

Table 2 shows the values of Haversian canal density in the cortex of the femur at different anatomical

Table 2. Haversian canal density in the femoral cortex depending on species, gender, age and anatomical location

Species	Gender	Age	Anatomical location	Haversian canal density (mm^2)	References
Homo sapiens	♀/♂	54.6 ± 21.2 years	shaft-subperiosteal area	12.3 ± 2.4	Cooper et al. 2007
Homo sapiens	♀	53.4 ± 23.6 years	shaft-subperiosteal area	11.5 ± 2.5	Cooper et al. 2007
Homo sapiens	♂	55.3 ± 19.9 years	shaft-subperiosteal area	12.7 ± 2.2	Cooper et al. 2007
Oryctolagus cuniculus	♂	8 months	middle shaft	111.3 ± 14.48	Pazzaglia et al. 2010
Oryctolagus cuniculus	♂	8 months	distal shaft	58.87 ± 16.93	Pazzaglia et al. 2010
Oryctolagus cuniculus	♂	8 months	shaft-subperiosteal area	125.1 ± 13.43	Pazzaglia et al. 2010
Oryctolagus cuniculus	♂	8 months	Shaft-subendosteal area	87.82 ± 46.98	Pazzaglia et al. 2010

locations in humans and rabbits, depending on sex and age. Haversian canal density in the femoral middle shaft in rabbits is higher than in the distal shaft, which is accordant with the fact that the presence of blood vessels in the middle shaft is higher than in the distal shaft (Pazzaglia et al. 2008, 2010). Additionaly, Haversian canal density is significantly higher in the subperiosteal than the subendosteal part of the cortex which is probably the result of intensive remodelling in the subperiosteal area (Pazzaglia et al. 2010).

Haversian canal density in rabbits is up to 10 times higher than in humans (Table 2). Since each osteon has one Haversian canal, a high density of Haversian canals in rabbit cortical bone indicates that the density of the osteon is large. Further, Haversian canal density in humans does not differ significantly with regard to sex (Table 2), which is consistent with the fact that the distance between Haversian canals does not vary significantly between the sexes in humans being 337.2 ± 38.3 μm in men and 53.1 ± 3.33 μm in women (Cooper et al. 2007).

2.1.4. Vascular canals in the femoral cortex

Table 3 presents data about the density of vascular canal openings, vascular canal density and diameter in rabbits with respect to age, gender and anatomical location. The same age and gender specimens have vascular canal densities much greater in the femoral middle shaft than in the distal shaft cortex (Pazzaglia et al. 2007). The density of Haversian canals is also greater in the femoral middle shaft (Table 1). Since the Haversian canal is occupied by blood vessels these two facts are in agreement. Differences in vascular canal densities in older and younger specimens occur due to remodelling processes that take place during aging. Vascular canal density is therefore greater in older specimens (Pazzaglia et al. 2007). Also, the vascular canal diameter in femoral cortical bone in rabbits corresponds to the average diameter of Haversian canals because the blood vessels are mostly situated there (Table 1 and 3).

The number of intracortical canal openings on cortical bone surface is directly correlated to the distribution of blood vessels located inside or outside the bone. Openings represent spots where blood vessels enter or exit bone. Scanning Electron Microscopy analysis showed that the number of canal openings on the endosteal surface in the middle shaft is larger than in the distal shaft which is not the case on the periosteal surface (Pazzaglia et al. 2009). This is probably because the inner side, which is closer to bone marrow, has more blood vessels that are entering the bone tissue, compared to the periosteal surface (Ohtani et al. 1982; Skawina et al. 1994). The density of vascular canal openings is the highest in the middle shaft and this is logical because canal density is also highest in this part of the bone. Canal openings on the endosteal surface in the middle shaft have

Table 3. Density of vascular canal openings, vascular canal density and diameter in the femoral cortex depending on species, gender, age and anatomical location

Species	Gender	Age (months)	Anatomical location	Density of vascular canal openings (mm^2)	Vascular canal density (mm^2)	Vascular canal diameter (μm)	References
Oryctolagus cuniculus	♂	8	middle shaft	_	142.3 ± 34.41	39 ± 2.4	Pazzaglia et al. 2007
Oryctolagus cuniculus	♂	8	distal shaft	_	84.13 ± 22.13	26.3 ± 5.0	Pazzaglia et al. 2007
Oryctolagus cuniculus	♂	1.5	middle shaft	_	112 ± 25.22	27 ± 1.46	Pazzaglia et al. 2007
Oryctolagus cuniculus	♂	1.5	distal shaft	_	91.25 ± 15.41	26.71 ± 2.89	Pazzaglia et al. 2007
Oryctolagus cuniculus	♂	8	middle shaft: periosteal surface	25.86 ± 4.84	–	–	Pazzaglia et al. 2009
Oryctolagus cuniculus	♂	8	distal shaft: periosteal surface	22.2 ± 5.6	–	–	Pazzaglia et al. 2009
Oryctolagus cuniculus	♂	8	middle shaft: endosteal surface	44.84 ± 19.6	–	–	Pazzaglia et al. 2009
Oryctolagus cuniculus	♂	8	distal shaft: endosteal surface	27.56 ± 4.41	–	–	Pazzaglia et al. 2009

an oval shape and diameters of between 30 μm and 1000 μm (Pazzaglia et al. 2009).

Five different shapes of canals were found in rabbit femoral cortical bone: cylindrical 39.2%, conical with proximal openings 9.4%, conical with distal openings 35.1%, elliptical 12.2% and hyperbolic 4.1% (Pazzaglia et al. 2010). Cooper et al. (2006) reported that cylindrical canals are located in primary osteons and in secondary osteons with lamelar apposition. Elliptical and hyperbolic canals are located at spots where irregular absorption occurs. Canals that are still in the process of maturing have conical shapes and the position of the widest opening is in the direction of the cutting cone (Cooper et al. 2006). The total volume of all canals compared to the total volume of bone tissue (in rabbit femoral cortical bone) is 5.88 ± 2.21% (Pazzaglia et al. 2010).

2.1.5. Lacuno-canalicular network

Osteocytes are found in cavities, called lacunae, of the osteons lamellae (Figure 1) and they communicate with each other via dendritic-like extensions that pass through micro-canals called canaliculi (Doty et al. 1981). Each osteocyte has dozens of thin dendritic extensions; for example, there are 41 in humans and 115 in horse (Beno et al. 2006). The Lacuno-canalicular network is the finest network in the cortical bone tissue which orientation and morphology depends on the species, age, anatomical location on the same bone, and arrangement of collagen fibres in the bone matrix and on effects of mechanical loads (Schneider et al. 2010). For example, it was found that the density of lacunae was reduced in older rats while the number of canaliculi increased and they became more properly organized (Okada et al. 2002). Some data have been reported regarding lacuno-canalicular geometry; we present some of these data below.

In mice, lacunae in long bones are generally elliptical in shape but they can be round also, while their volume varies from 191 μm^3 to 274 μm^3 and their mean space is 26 μm (Wang et al. 2005; Schneider et al. 2010) However, in rabbit long bones lacunar volumes are about 350 μm^3 (Remaggi et al. 1998). Lacunae are parallel to the axis of the bone and it is believed that this is due to the influence of mechanical loads (Vatsa et al. 2008).

Canaliculi are cylindrical in shape and they are arranged asymmetrically along the lacunar canal, gradually increasing its diameter towards it (You et al. 2004). In several different animal species and in humans, it was found that the number of canaliculi is higher on the side of the lacunae that face the Haversian canal than on the side that face the cement line, so this might be a pattern (Remaggi et al. 1998). This way of arrangement of canaliculi is probably caused by different distance from the blood vessels (Schneider et al. 2010). In mice, the average diameter of canaliculi is 259 nm while the average length is 104 nm (You et al. 2004).

2.1.6. Cortical bone thickness in the femur

Table 4 shows data regarding cortical bone thickness in the femur in humans, rabbits and rats regarded to age, gender and anatomical location. Sex hormones have a significant impact on cortical bone thickness as well as on the bone microarchitecture (Bagi et al. 1997; Tromp et al. 2006). The average thickness of cortical bone located in the femoral neck region in postmenopausal women is about 10 times greater compared to ovariectiomised female rats (Bagi et al. 1997). Also, cortical thickness in intact women is approximately 10 times greater than in intact rabbits. Since, cortical bone thickness represents a macro-structural entity it is not appropriate to compare these data because humans have bigger femurs than rats and rabbits, so cortical bone thickness is also proportionally greater. However, it was shown that cortical bone on cross section occupies 72.5% of the total bone area in the rat femur (Figure 2), while in humans it is only 12.5% (Bagi et al. 1996, 1997). Differences in corti-

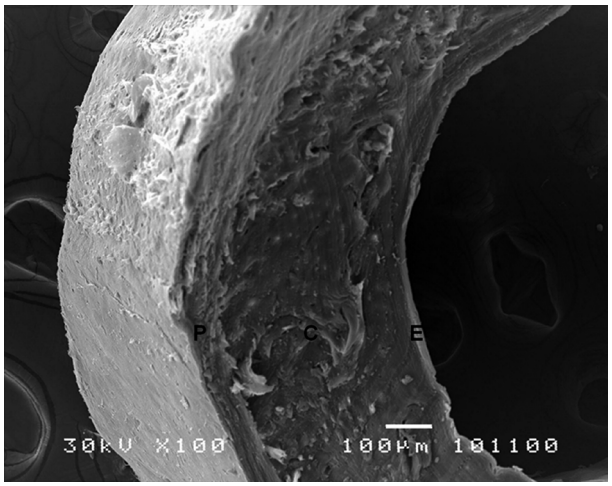

Figure 2. Femoral cross section in rat *Rattus norvegicus* (Wistar strain). P = periosteum; C = cortex; E = endosteum. Scanning Electron Micrography; bar 100 μm

Table 4. Cortical bone thickness in the femur depending on species, gender, age and anatomical location

Species	Gender	Age	Anatomical location	Cortical bone thickness (mm)	References
Homo sapiens (postmenopasual)	♀	66.5 years	neck	3.3 ± 1.4	Bagi et al. 1997
Homo sapiens	♀/♂	20–29 years	shaft	6.06 ± 1.0	Jowsey 1966
Homo sapiens	♀/♂	50–59 years	shaft	5.92 ± 0.8	Jowsey 1966
Homo sapiens	♀/♂	54.6 ± 21.2 years	shaft	4.68 ± 1.18	Cooper et al. 2007
Homo sapiens	♀	53.4 ± 23.6 years	shaft	4.01 ± 1.23	Cooper et al. 2007
Homo sapiens	♂	55.3 ± 19.9 years	shaft	5.06 ± 0.97	Cooper et al. 2007
Ratus norvegicus (ovariectomized)	♀	12 months	neck	0.3 ± 0.01	Bagi et al. 1997
Oryctolagus cuniculus	♂	8 months	middle shaft	0.456 ± 0.06	Pazzaglia et al. 2007
Oryctolagus cuniculus	♂	8 months	distal shaft	0.606 ± 0.12	Pazzaglia et al. 2007
Oryctolagus cuniculus	♂	1.5 months	middle shaft	0.584 ± 0.09	Pazzaglia et al. 2007
Oryctolagus cuniculus	♂	1.5 months	distal shaft	3.3 ± 1.4	Pazzaglia et al. 2007

cal bone abundance are due to a specific response to different mechanical loads and strains in humans and rats bones during locomotion (Martens et al. 1983). The femur in humans, especially the femoral neck, is exposed to higher mechanical load during bipedal locomotion than the femur in rats. Because of that cancellous tissue occupies a bigger part than cortical bone in the human femur and vice versa in rats, which will be discussed later. Further, in rabbits and rats differences between cortical bone thickness in distal and middle femoral parts are much smaller; this is not the case in humans (Table 4) (Pazzaglia et al. 2007).

2.2. Microarchitecture of cancellous bone tissue

Cancellous bone is a sponge-like bone and it is made out of elements called trabeculae, which look like honeycomb, and differ in size and shape (Buckwalter et al. 1996). Cancellous bone is the most abundant in the metaphyseal area of long bones and it is surrounded by cortical bone. The porosity of trabecular bone in intact rats is between 50 to 90%; the same is broadly true in human (Buckwalter et al. 1996; Sikavitsas et al. 2001; Yang et al. 2011). Osteocyte density in cancellous bone in the femoral head (*caput femoris*) in rats is 93 000 mm^{-3}, and in mice from 50 000 mm^{-3} to 66 000 mm^{-3} in the femoral middle shaft (Mullender et al. 1996; Schneider et al. 2007)

Data on the thickness, space and number of trabeculae in humans and rats with respect to age, gender and anatomical location are shown in Table 5. There is a significant difference in trabecular geometry between ovariectomised and intact female rats. Trabeculae lose their thickness and become less abundant while the trabecular space increases. Aging is one of the factors that contribute to this process, too (Bagi et al. 1996). In humans trabecular thickness and space is very different between the tibia and femur, while the abundance of trabeculae is similar, which is not the case in rats (Table 5). Trabeculae in the femoral neck are approximately four times thicker in humans than in rats, and because of that there are less trabeculae per 1 mm^2 in humans than in rats. This difference occurs also because of the unequal femoral size among these two species meaning that macro-structural entities cannot be compared. However, it was shown that in rats cancellous bone tissue occupies a proportionally smaller area than in humans (Bagi et al. 1997). Despite this, humans have more cancellous bone tissue which increases the total strength of the femur and acts like a load conductor, allowing bipedal locomotion (Wolff 1892; Pugh et al. 1973; Martens et al. 1983; Parfitt 1984 1987). In addition, humans have two kinds of cancellous bone tissue: peripheral and central part of the cancellous bone. Peripheral cancellous bone is organised as a net of plate-like trabeculae which are oriented in the direction of body weight forces, and are connected with rod-like perpendicular trabeculae. The central part of spongy bone consists mostly of rod-like trabeculae (Bagi et al. 1997).

Changes in sex hormone levels in an organism are one of the most common causes of trabecular

Table 5. Trabecular thickness, space and number in the femur and tibia depending on species, gender, age and anatomical location

Species	Gender	Age	Anatomical location	Trabecular thickness (μm)	Trabecular space (μm)	Trabecular number (mm)	References
Homo sapiens	♀/♂	–	femoral methaphysis	128.6–201.0	313.5–619.4	1.34–1.94	Nogueira et al. 2010
Homo sapiens	♀/♂	67.3 ± 15.5 years	femoral head	127–284	454–940	1.092–2.387	Hildebrand et al. 1999
Homo sapiens	♀/♂	69 ± 15 years	femoral head	120–257	480–984	0.98–1.91	Ulrich et al. 1999
Homo sapiens	♀/♂	–	tibial methaphysis	379.5–372.4	211.7–131.1	1.69–1.99	Nogueira et al. 2010
Ratus norvegicus (ovariectomized)	♀	12 months	femoral methaphysis	62.7 ± 3.2	386.3 ± 34.2	4.1 ± 0.4	Bagi et al. 1997
Ratus norvegicus (ovariectomized)	♀	12 weeks	femoral methaphysis	102 ± 12	130 ± 39	4.4 ± 0.6	Tromp et al. 2006
Ratus norvegicus	♀	12 weeks	femoral methaphysis	91 ± 14	93 ± 20	5.5 ± 0.7	Tromp et al. 2006
Ratus norvegicus	♀/♂	–	proximal tibia	48.2–64.1	158.3–188.1	5.2–5.8	Hanson and Bagi 2004
Ratus norvegicus	♀	6 months	proximal tibia	50	–	4.01	Mavropoulos et al. 2007

structural variation (Bagi et al. 1996; Mavropoulos et al. 2007; Yang et al. 2011). Ovariectomy in female rats causes a loss in trabecular tissue volume of 82% and reduces the number of trabeculae by 63% in proximal tibia during the first 17 weeks after the ovariectomy (Mavropoulos et al. 2007). In ovariectomised females trabecular tissue is four times less abundant than in control groups and mineral density in trabeculae significantly decreases (Bagi et al. 1996; Yang et al. 2011). Also, a loss of bone volume of 76% and trabecular abundance of 56% in proximal tibia region was reported in rats that were on a protein-restricted diet (Mavropoulos et al. 2007). However, low levels of sex hormones and a protein-restricted diet has more negative effects on cancellous tissue volume and the number of trabeculae in proximal tibia bone than in rat mandibulae (Mavropoulos et al. 2007). One explanation is that during mastication cancellous bone tissue in the mandibula is exposed to a higher mechanical load than the tibial bone during locomotion. In addition, a control group, which was fed with soft food, had greater loss in cancellous tissue of the mandibula compared to the group which was fed with solid food (Mavropoulos et al. 2007). Similar parameters of cancellous bone in female rats were investigated, and it was shown that loads in backpacks compensate the negative effects of a lack of sex hormones on cancellous bone. Additionally, mechanical load produced by backpack loads increased bone mineral content and bone mineral density by 27% and 11% respectively, comparing to control groups (Tromp et al. 2006). The same conclusion was made in experiments dealing with astronauts in zero gravity conditions and in other similar studies (Vico et al. 2000; Shackelford et al. 2004). Also, it was shown that intense physical activity in humans preserves trabecular bone microarchitecture by increasing the number of trabeculae in tibia bone by 12.7% compared to the control group (Nilsson et al. 2010).

3. Concluding remarks

In conclusion, we can say that the structure of bone tissue is very specific and varies during life, and in different functional and physiological stages. There is no single factor which independently affects bone structure and observed effects are rather due to a specific combination of many factors. However, it is our opinion that mechanical loading has a stronger impact on bone microarchitecture at all levels in comparison with other factors. For example osteon wall thickness is greater in younger persons than in older ones, since Haversian canal diameter is smaller and osteon diameter is bigger.

But this regularity was not noticed in the cortical bones of ribs so it could not be the underlying reason for why these parameters are changing. This is probably because the mechanical loading of ribs is constant during life, which is not the case in weight-bearing bones like the femur or tibia. In addition, overweight persons in the same age group, have thicker osteon walls. Higher loads also trigger osteon diameter reduction due to physical activity or higher body weight. The biological significance of such processes and changes is an increase in bone strength, and also increasing resistance to micro- and macro-fractures. Also, osteon wall thickness and osteon density are higher in rabbits compared to rhesus monkeys and humans because of different ways of locomotion and lifestyles. Therefore, rabbits have a more solid and stronger cortical bone that resists high impact force acting upon the femur during jumping. Furthermore, it was shown that a significant correlation exists between morphological parameters of bone tissue and its mechanical properties – strength and stiffness (Voide et al. 2008).

Osteon orientation in long bones is also affected by mechanical loads and their orientation is parallel with direction of the maximum principal stress and strain (Lanyon and Bourn 1979; Fiala and Hert 1993; Hert et al. 1994; Baca et al. 2007). They form two helical systems, opposite one another, located at opposite sides of the diaphysis of the long bone. One of these systems is always bigger than the other one because of torque moments. This osteon orientation relates to loading forces during lifetime mostly during motion and other life activities (Hert et al. 1994). This pattern is seen to be widespread in long bones of lower limbs in mammals, which was confirmed by many different studies (Cohen and Harris 1958; Fiala and Hert 1993; Hert et al. 1994; Mohsin et al. 2002; Baca et al. 2007).

Differences in cortical bone tissue and cancellous bone tissue between humans and rats are great. The femur is heavily loaded bone in humans, especially in the metaphyseal region; consequently cancellous bone tissue is more developed in this region compared to rats, because cancellous bone conducts mechanical forces better than cortical bone and acts like an armature and load absorber. Variations in bone tissue structure are strongly influenced by different levels of sex hormones, but intense load could preserve bone tissue volume despite low level of sex hormones (Tromp et al. 2006; Mavropoulos et al. 2007).

For successful treatment of bone defects it is very important to integrate the basic elements in bone tissue engineering – cells and biomaterial scaffolds – with patient gender, age, health, anatomical shape, position and microarchitecture of the bone defect. Therefore, all parameters mentioned above, including morphological ones, must be considered in such treatments. This is especially important when bone defects are being treated with bone substituents which must be compatible in every sense with the bone itself. Bone substituents with adequate microarchitecture enable angiogenesis and osteogenic cell propagation, which is very important for osteoreparative and osteoregenerative processes as we have already shown (Ajdukovic et al. 2005; Janicijevic et al. 2008; Vasiljevic et al. 2009). In that sense this overview could be of benefit in further studies and for the fabrication of artificial bone substituents for bone tissue engineering.

Commonly used animal models (i.e. rats and rabbits) are very important in preclinical studies of various bone tissue defect healing. Because there is not an ideal animal model, an understanding of the differences in bone microarchitecture between humans and animal models is needed for extrapolation and interpretation of the results from *in vivo* studies. We hope that in this regard the present review will be of benefit. Also, a proper experimental set up and choice of suitable animal models is crucial. Therefore, this overview should be helpful in facilitating decisions on which animal models are most suitable for different preclinical studies.

4. REFERENCES

Ajdukovic Z, Najman S, Dorđevic L, Savic V, Mihailovic V, Petrovic D, Ignjatovic N, Uskokovic D (2005): Repair of bone tissue affected by osteoporosis with hydroxyapatite-poly-L-lactide (HAp-PLLA) with and without blood plasma. Journal of Biomaterials Applications 20, 179–190.

Ardizzoni A (2001): Osteocyte lacunar size-lamellar thickness relationships in human secondary osteons. Bone 28, 215–219.

Baca V, Kachlik D, Horak Z, Stingl J (2007): The course of osteons in the compact bone of the human proximal femur with clinical and biomechanical significance. Surgical and Radiologic Anatomy 29, 201–207.

Bagi C, DeLeon E, Ammann P, Rizzoli R, Miller S (1996): Histo anatomy of the proximal femur in rats, Impact

of ovariectomy on bone mass, structure, and stiffness. Anatomical Record 245, 633–644.

Bagi C, Wilkie D, Georgelos K, Williams D, Bertolini D (1997): Morphological and structural characteristics of the proximal femur in human and rat. Bone 21, 261–267.

Beno T, Yoon YJ, Cowin SC, Fritton SP (2006): Estimation of bone permeability using accurate microstructural measurements. Journal of Biomechanics 39, 2378–2387.

Britz HM, CDL Thomas CDL, JG Clement JG, Cooper DML (2009): The relation of femoral osteon geometry to age, sex, height and weight. Bone 45, 77–83.

Buckwalter J, Glimcher M, Cooper R, Recker R (1996): Bone biology. I. Structure, blood supply, cells, matrix, and mineralization. Instructional Course Lectures 45, 371–386.

Burr DB, Ruff CB, Thompson DD (1990): Patterns of skeletal histologic change through time, comparison of an archaic Native American population with modern populations. Anatomical Record 226, 307–313.

Cohen J, Harris WH (1958): The three-dimensional anatomy of Haversian systems. Journal of Bone and Joint Surgery 40, 419–434.

Cooper DML, Thomas CDL, Clement JG, Hallgrímsson B (2006): Three-dimensional microcomputed tomography imaging of basic multicellular unit-related resorption spaces in human cortical bone. Anatomical Record Part A 288, 806–816.

Cooper DML, Thomas CDL, Clement JG, Turinsky AL, Sensen CW, Hallgrimsson B (2007): Age-dependent change in the 3D structure of cortical porosity at the human femoral midshaft. Bone 40, 957–965.

Currey JD (1964): Some effects of ageing in human Haversian systems. Journal of Anatomy 98, 69–75.

Doty SB (1981): Morphological evidence of gap junctions between bone cells. Calcified Tissue International 33, 509–512.

Fiala P, Hert J (1993): Principal types of functional architecture of cancellous bone in man. Functional and Developmental Morphology 3, 91–99.

Frost H (1990): Skeletal structural adaptations to mechanical usage (SATMU). 2. Redefining Wolff's law, the remodeling problem. Anatomical Record 226, 414–422.

Hanson NA, Bagi CM (2004): Alternative approach to assessment of bone quality using micro-computed tomography. Bone 35, 326–333.

Harris WH, Haywood EA, Lavorgna J, Hamblen DL (1968): Spatial and temporal variations in cortical bone formation in dogs, a long-term study. Journal of Bone and Joint Surgery 50, 1118–1128.

Havill L (2003): Osteon remodeling dynamics in Macaca mulatta, normal variation with regard to age, sex, and skeletal maturity. Calcified Tissue International 74, 95–102.

Hert J, Fiala P, Petrtyl M (1994): Osteon orientation of the diaphysis of the long bones in man. Bone 15, 269–277.

Hildebrand T, Laib A, Muller R, Dequeker J, Ruegsegger P (1999): Direct three dimensional morphometric analysis of human cancellous bone, microstructural data from spine, femur, iliac crest, and calcaneus. Journal of Bone and Mineral Research 14, 1167–1174.

Janicijevic JM, Najman SJ, Ignjatovic NL, Savic VP, Kocic JS, Vasiljevic PJ, Vukelic MD, Uskokovic DP (2008): Nanomaterial N-CP/DLPLG as potentional tissue graft in osteoreparation in combination with bone marrow cells on subcutaneous implantation model. Hemijska Industrija 62, 205–210.

Jowsey J (1964): Variations in bone mineralization with age and disease. Bone Bio 461–479.

Jowsey J (1966): Studies of Haversian systems in man and some animals. Journal of Anatomy 100, 857–864.

Kalicanin BM, Ajdukovic ZR, Kostic M, Najman SJ, Savic VP, Ignjatovic NL (2007): The role of synthetic biomaterials in resorptive alveolar bone regeneration. Hemijska Industrija 61, 96–100.

Lanyon LE, Bourn S. (1979): The influence of mechanical function on the development and remodeling of the tibia. An experimental study in sheep. Journal of Bone and Joint Surgery 61, 263–273.

Lee WR, JH Marshall JH, Sissons HA (1965): Calcium accretion and bone formation in dogs. An experimental comparison between the results of Ca-45 kinetic analysis and tetracycline labelling. Journal of Bone and Joint Surgery 47, 157–180.

Levick A (2004): Netter's concise atlas of orthopaedic anatomy. Australasia Chiropractic and Osteopathy 12, 86.

Marks SC, Hermey DC (1996): The structure and development of bone. In: Bilezikian JP, Raisz LG, Rodan GA (eds.): Principles of Bone Biology. Academic Press, San Diego. CA. 3–14.

Marotti G (1996): The structure of bone tissues and the cellular control of their deposition. Italian Journal of Anatomy and Embryology 101, 25–79.

Marotti G, Muglia M (1988): A scanning electron microscope study of human bony lamellae. Proposal for a new model of collagen lamellar organization. Italian Journal of Anatomy and Embryology 93, 163–175.

Martens M, Audekercke RV, Delport P, Meester PD, Mulier J (1983): The mechanical characteristics of cancellous bone at the upper femoral region. Journal of Biomechanics16, 971–983.

Martin RB, JC Pickett JC, Zinaich S (1980): Studies of skeletal remodeling in aging men. Clinical Orthopaedics and Related Research 149, 268–282.

Martin RB, Burr DB, Sharkey NA (1998): Skeletal Tissue Mechanics. Springer Verlag. 392 pp.

Martiniakova M, Omelka R, Grosskopf B, Sirotkin A, Chrenek P (2008): Sex-related variation in compact bone microstructure of the femoral diaphysis in juvenile rabbits. Acta Veterinaria Scandinavica 50, 15.

Mavropoulos A, Rizzoli R, Ammann P (2007): Different responsiveness of alveolar and tibial bone to bone loss stimuli. Journal of Bone and Mineral Research 22, 403–410.

Mohsin S, Taylor D, Lee T (2002): Three-dimensional reconstruction of Haversian systems in ovine compact bone. European Journal of Morphology 40, 309–315.

Mohsin S, O'Brien F, Lee T (2006): Osteonal crack barriers in ovine compact bone. Journal of Anatomy 208, 81–89.

Mulhern DM, Ubelaker DH (2009): Bone microstructure in juvenile chimpanzees. American Journal of Physical Anthropology 140, 368–375.

Mullender M, Huiskes R, Versleyen H, Buma P (1996): Osteocyte density and histomorphometric parameters in cancellous bone of the proximal femur in five mammalian species. Journal of Orthopaedic Research 14, 972–979.

Najman S, Savic V, Djordjevic L, Ignjatovic N, Uskokovic D (2004): Biological evaluation of hydroxyapatite/poly-L-lactide (HAp/PLLA) composite biomaterials with poly-L-lactide of different molecular weights intraperitoneally implanted into mice. Biomedical Materials and Engineering 14, 61–70.

Nilsson M, Ohlsson C, Sundh D, Mellstrom D, Lorentzon M (2010): Association of physical activity with trabecular microstructure and cortical bone at distal tibia and radius in young adult men. Journal of Clinical Endocrinology and Metabolism 95, 2917–2926.

Nogueira L, Braz D, Barroso R, Oliveira L, Pinheiro C, Dreossi D, Tromba G (2010): 3D histomorphometric quantification of trabecular bones by computed microtomography using synchrotron radiation. Micron 41, 990–996.

Ohtani O, Gannon B, Ohtsuka A, Murakami T (1982): The microvasculature of bone and especially of bone marrow as studied by scanning electron microscopy of vascular casts – a review. Scanning Electron Microscopy (Pt 1), 427–434.

Okada S, Yoshida S, Ashrafi SH, Schraufnagel DE (2002): The canalicular structure of compact bone in the rat at different ages. Microscopy and Microanalysis 8, 104–115.

Parfitt A (1984): Age-related structural changes in trabecular and cortical bone, cellular mechanisms and biomechanical consequences. Calcified Tissue International 36, 123–128.

Parfitt AM (1987): Trabecular bone architecture in the pathogenesis and prevention of fracture. American Journal of Medicine 82, 68–72.

Parfitt A (1994): Osteonal and hemi osteonal remodelling: The spatial and temporal framework for signal traffic in adult human bone. Journal of Cellular Biochemistry 55, 273–286.

Pazzaglia U, Bonaspetti G, Rodella LF, Ranchetti F, Azzola F (2007): Design, morphometry and development of the secondary osteonal system in the femoral shaft of the rabbit. Journal of Anatomy 211, 303–312.

Pazzaglia U, Bonaspetti G, Ranchetti F, Bettinsoli P (2008): A model of the intracortical vascular system of long bones and of its organization, an experimental study in rabbit femur and tibia. Journal of Anatomy 213, 183–193.

Pazzaglia U, Congiu T, Raspanti M, Ranchetti F, Quacci D (2009): Anatomy of the intracortical canal system, scanning electron microscopy study in rabbit femur. Clinical Orthopaedics and Related Research 467, 2446–2456.

Pazzaglia U, Zarattini G, Giacomini D, Rodella L, Menti A, Feltrin G (2010): Morphometric analysis of the canal system of cortical bone, an experimental study in the rabbit femur carried out with standard histology and micro CT. Anatomia Histologia Embryologia 39, 17–26.

Pearce A, Richards R, Milz S, Schneider E, Pearce S (2007): Animal models for implant biomaterial research in bone, a review. European Cells and Materials Journal 13, 1–10.

Pfeiffer S (1998): Variability in osteon size in recent human populations. American Journal of Physical Anthropology 106, 219–227.

Podshivalov L, Holdstein Y, Fisher A, Bar-Yoseph PZ (2008): Towards a multi-scale computerized bone diagnostic system, 2D micro-scale finite element analysis. Communications in Numerical Methods in Engineering 25, 733–749.

Pugh J, Rose R, Radin E (1973): Elastic and viscoelastic properties of trabecular bone, dependence on structure. Journal of Biomechanics 6, 475–478.

Remaggi F, Cane V, Palumbo C, Ferretti M (1998): Histomorphometric study on the osteocyte lacuno-canalicular network in animals of different species. I. Woven-fibered and parallel-fibered bones. Italian Journal of Anatomy and Embryology 103, 145–155.

Ross MH, Reith EJ, Romrell LJ (1989): Histology, a text and atlas. 4th ed. Williams and Wilkins, Baltimore.

Schaffler MB, Burr DB (1984): Primate cortical bone microstructure, relationship to locomotion. American Journal of Physical Anthropology 65, 191–197.

Scheuer L (2002): Application of osteology to forensic medicine. Clinical Anatomy 15, 297–312.

Schneider P, Stauber M, Voide R, Stampanoni M, Donahue LR, Muller R (2007): Ultrastructural properties in corti-

cal bone vary greatly in two inbred strains of mice as assessed by synchrotron light based micro and nano CT. Journal of Bone and Mineral Research 22, 1557–1570.

Schneider P, Meier M, Wepf R, Müller R (2010): Towards quantitative 3D imaging of the osteocyte lacuno-canalicular network. Bone 47, 848–858.

Shackelford L, Leblanc A, Driscoll T, Evanis H, Rianon N, Smith S, Spector E, Feeback D, Lai D (2004): Resistance exercise as a countermeasure to disuse-induced bone loss. Journal of Applied Physiology 97, 119–129.

Sikavitsas VI, Temenoff JS, Mikos AG (2001): Biomaterials and bone mechanotransduction. Biomaterials 22, 2581–2593.

Singh I. Gunberg D (1971): Quantitative histology of changes with age in rat bone cortex. Journal of Morphology 133, 241–251.

Skawina A, Litwin J, Gorczyca J, Miodonski A (1994): The vascular system of human fetal long bones, a scanning electron microscope study of corrosion casts. Journal of Anatomy 185, 369–376.

Sugawara Y, Kamioka H, Honjo T, Tezuka K, Takano-Yamamoto T (2005): Three-dimensional reconstruction of chick calvarial osteocytes and their cell processes using confocal microscopy. Bone 36, 877–883.

Takahashi H, Epker B, Frost H (1965): Relation between age and size of osteons in man. Henry Ford Hospital Medical Bulletin 13, 25–31.

Thompson D (1981): Microscopic determination of age at death in an autopsy series. Journal of Forensic Sciences 26, 470–475.

Tromp A, Bravenboer N, Tanck E, Oostlander A, Holzmann P, Kostense P, Roos J, Burger E, Huiskes R, Lips P (2006): Additional weight bearing during exercise and estrogen in the rat, the effect on bone mass, turnover, and structure. Calcified Tissue International 79, 404–415.

Ulrich D, van Rietbergen B, Laib A (1999): The ability of three-dimensional structural indices to reflect mechanical aspects of trabecular bone. Bone 25, 55–60.

Urbanova P, Novotny V (2005): Distinguishing between human and non-human bones, histometric method for forensic anthropology. Anthropologie 43, 77–85.

van Oers RFM, Ruimerman R, Rietbergen van B, Hilbers PAJ, Huiskes R (2008): Relating osteon diameter to strain. Bone 43, 476–482.

Vasiljevic PJ, Najman SJ, Dorđevic LB, Savic VP, Vukelic MĐ, Zivanov-Curlis JZ, Ignjatovic NL, Uskokovic DP (2009): Ectopic osteogenesis and hematopoiesis after implantantion of bone marrow cells seeded on HAP/PLLA scaffold. Hemijska Industrija 63, 301–307.

Vatsa A, Breuls RG, Semeins CM, Salmon PL, Smit TH, Klein-Nulend J (2008): Osteocyte morphology in fibula and calvaria: Is there a role for mechanosensing? Bone 43, 452–458.

Vico L, Collet P, Guignandon A, Lafage-Proust MH, Thomas T, Rehailia M, Alexandre C (2000): Effects of long-term microgravity exposure on cancellous and cortical weight-bearing bones of cosmonauts. Lancet 355, 1607–1611.

Voide R, van Lenthe GH, Muller R (2008): Bone morphometry strongly predicts cortical bone stiffness and strength, but not toughness, in inbred mouse models of high and low bone mass. Journal of Bone and Mineral Research 23, 1194–1203.

Vukelic M, Najman S, Zivkovic J, Stanisavljavic M, Cvetkovic V, Najdanovic J, Zivanov-Curlis J (2011): The role of periferal blood cells in the induction of ectopic osteogenesis. In: Thirteenth Annual Conference – YUCOMAT, Herceg Novi, Montenegro, 5–9 September 2011, Programme and the Book of Abstracts, 174 pp.

Wang L, Wang Y, Han Y, Henderson SC, Majeska RJ, Weinbaum S, Schaffler MB (2005): In situ measurement of solute transport in the bone lacunar-canalicular system. Proceedings of the National Academy of Sciences of the United States of America 102, 11911–11916.

Wolff J (1892): The Law of Bone Transformation. Hirschwald, Berlin.

Yang X, Chan YH, Muthukumaran P, DasDe S, Teoh SH, Lee T (2011): Ibandronate does not reduce the anabolic effects of PTH in ovariectomized rat tibiae, A microarchitectural and mechanical study. Bone 48, 1154–1163.

You LD, Weinbaum S, Cowin SC, Schaffler MB (2004): Ultrastructure of the osteocyte process and its pericellular matrix. Anatomical Record Part A 278, 505–513.

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Corresponding Author:

Vladimir J. Cvetkovic, University of Nis, Faculty of Sciences and Mathematics, Department of Biology and Ecology, Visegradska, 33, 18000 Nis, Serbia

Tel. +381 1835 5015, E-mail: biovlada@yahoo.com