Fracture healing; CT; quantitative assessment; image analysis, ultrasound, ultrasonometry, Computer Aided Diagnosis

Wojciech GLINKOWSKI *,**,****, Jarosław Żyłkowski****,
Artur WOJCIECHOWSKI[***, ****], Andrzej GÓRECKI[**]

THE DEVELOPMENT OF METHODS FOR QUANTITATIVE CLINICAL ASSESSMENT OF THE FRACTURE HEALING

The quantitative measurement is required for fracture healing evaluation in clinical studies approaching toward Evidence Based Medicine. Realistic and quantitative evaluation of fracture healing may create a new era for orthopedic trauma science. The analysis of various factors, including biological, pharmacological, physical, mechanical, genetic and others, influence on fracture healing remains great challange in orthopedic medicine.
A reliable method should allow the determination time of adequate union by its strength, quality and quantity, but the busy orthopedic surgeon require suitable methods with user friendly interface to implement them into daily clinical practice. Authors describe various methods including radiography, Computed Tomography, Ultrasound imaging, Ultrasonometry and Computer Aided Diagnosis (RODIA, 3D Reconstructor). Most accurate methods as histology or mechanical testing are suitable in experimental studies less likely used clinically. Clinically validated and tested methods usually utilize X-ray and CT data. Computer Aided Diagnostics may enhance and support managing the patients, clinical trials and practical applications.

1. INTRODUCTION

There is a lack of consensus in the assessment of fracture healing of long bones fractures among orthopedic surgeons. The decision to intervene in an effort to promote fracture healing may be influenced by varying definitions of nonunion and delayed union. The daily practice assessment of fracture healing is mostly a clinically relevant.
Subjective physical examination of bone union may not lead to correct conclusions, so an assessment using a more objective method of analyzing the degree of healing of a fracture is required for the identification of the completion of fracture healing, and treatment discontinuation. Histological evaluation is considered a method finally determining diagnosis however as an invasive method it may not be applicable in most clinical cases.
Definite measurements may lead to improve refractures prediction, healing monitoring, and rationale treatment. This would provide quantitative assessment of the fracture healing progress. The analysis of how various factors including

[*] Department of Anatomy, Center of Biostructure, Medical University of Warsaw, 02-004 Chałubińskiego 5, Poland
[**] Chair and Department of Orthopedics and Traumatology of the Locomotor System, Center of Excellence "TeleOrto", Medical Univeristy of Warsaw, 02-005 Lindleya 4, Poland
[***], Department of Clinical Radiology, Medicial University of Warsaw, 02-004 Chałubińskiego 5, Poland
[****] Polish Telemedicine Society

biological, pharmacological, physical, mechanical, genetic and others influence fracture healing may be evaluated with precise quantitative measurements of the healing quality and duration.

Fracture healing is a fundamental, specialized process leading to restoration of bone integrity and function. Repair impairment leads to delayed union or nonunion. Almost 10% of all of the fractures annually will require further interventions (including surgical) because of healing disturbances. The early identification of impaired healing patterns may result in the alteration of treatment to introduce as early as possible the optimal treatment leading to recovery instead of delayed or non union. Quantitative methods evaluation of fracture healing is required for Evidence Based Medicine. Surgeon's experience dependent clinical evaluation of bone union is mostly utilized in practice. Dating of fracture healing on the basis of radiographic appearance is rarely reported in the literature. Many articles on fracture repair and dating are derived from animal studies focusing on histological changes.

Fracture repair initiates immediately after the fracture and consists of three overlapping stages: inflammatory phase (5–7 days), reparative phase (4–40 days), and remodeling (months to years) [76].

The reparative phase encompasses radiographic visualization (fracture line changes, callus formation) of "normal fracture healing" with some overlap into the remodelling phase. However, many factors, including patient status, tissue variables, and treatment method can alter this sequence of events and influence the time when one would expect to see the first radiological signs of healing.

All current methods have limitations in reliability, artifacts, radiation hazards, subjectivity, or costs. This fact often leads to a delay in the diagnosis of impaired fracture healing, and consequently, to a delay in starting appropriate treatment, and contributes to the difficulties in managing impaired fracture healing.

Perfect measurable definitions of union, delayed union, nonunion, pseudoarthrosis, refracture does not exist. The optimal, noninvasive method should allow to: determine time of bone union, time for hardware removal, time of loading, prediction of impairment, effectiveness of treatment and influence of various factors.

The aim of this study is to overview methods developed for quantitative clinical assessment of the fracture healing useful in clinical practice and clinical trials including fracture healing enhancement methods [17, 30, 48, 52, 59, 63, 119].

Ultrasonometry

Ultrasound is widely used in medicine because of its diagnostic potential and safety. Ultrasonometric measurements are non-destructive utilizing no ionizing radiation. The ultrasonic measurement of bone has gained great interest due to its potential to reflect material, mechanical and structural properties. Through-transmission mode of ultrasonic examination is widely used for bone quality assessment. The primary interest in recent years has focused on the ultrasonic wave propagation through bones to assess of osteoporosis [12, 13, 18, 41, 42, 61, 62, 65, 84, 83, 88, 91, 117]. In the context of bone healing, the ultrasonometric measurements have the potential to monitor the course of healing, detect healing progress inhibition (e.g., delayed unions or nonunions) and quantitatively assess bone union.

Ultrasound has been employed as a monitoring tool in fracture healing [1- 3, 37-39, 42, 68-70, 92, 106]. The velocity of ultrasound wave propagation utilizing so-called axial-transmission technique is used as an indicator of healing. The technique applications have been found to be suitable to examining long bones. However, the pattern of velocity evolution as healing progresses has not been quantified, and no distinction has been made between partially healed bones and nonunions. Major disadvantages of the percutaneous applications are that the overlying soft tissues affect the repeatability and accuracy of the measurements and that they are only applicable to peripheral skeletal sites. Tibia, ulna and partially radius are anatomically well exposed to ultrasonometric examination.

The soft-tissue thickness, cortical bone thickness and fracture gap width, depth [67, 91, 120] and particularly the quality of the material within the fracture gap and fracture callus influence on FAS wave velocity. Transducers are rarely implanted and located on the bone surfaces in the vicinity of the fracture site [70, 96].

When the wavelength is comparable to, or smaller than, the thickness of the bone cortex, the FAS corresponds to a lateral (or head) wave propagating along the bone subsurface at the bulk wave velocity. Therefore, the first-arriving signal of the ultrasonic wave propagates mainly through superficial layer of the bone just underneath the periosteum. When the wavelength is comparable to or greater than the cortical thickness, the tubular bone geometry acts as a waveguide supporting the generation and propagation of additional wave modes.

Broadband attenuation, important for trabecular bone assessment was rarely mentioned in ultrasonic fracture healing monitoring literature.

The transmitter and receiver are placed on each side of the fracture site at exactly known distance between them. The transducers (transmitter and receiver) are placed on the skin on each side of the fracture site. The transit time of the first-arriving ultrasound wave is measured and the result is divided by transmitter-receiver distance. The velocity of ultrasound waves propagating along the long axis of bone is determined by the first-arriving signal (FAS) [43, 97]. Animal [1 ,36] and clinical studies [2, 34, 75, 38-40, 42, 43] have demonstrated that the propagation velocity across fractured bones gradually increases during healing. The examination requires measuring the contralateral intact bone to compare the result. Previous investigations clinical [2, 3, 25, 34, 38-40, 42, 43, 106] and experimental [1, 36] have demonstrated that the achieved bone union is represented by the velocity that equals or exceeds 80% of the contralateral intact bone.

Typically used devices for fracture healing monitoring operate frequencies in the range from 0.2 MHz to 2.5 MHz. Protopappas et al. [96, 97] have recently introduced, successfully tested and validated an integrated telemedicine system for both the monitoring and enhancement of fracture healing in long bones [70, 96]. They implanted a pair of ultrasound transducers into the fracture region, and a wearable device which wirelessly communicates data to a centralized data storage unit.

The system is designed for monitoring of the healing process in long bones. A pair of ultrasound transducers is mounted on a frame with fixed distance between transducers. The transducers contact with the skin directly above the examined bone on both sides of the fracture site. Fracture healing monitoring uses axial transmission mode. The circular transducers generate and receive longitudinal waves at their resonant frequency (1 MHz, 70% broadband). The device is interconnected to the PC for the transfer of the acquired data. The system collects effective and reliable ultrasound measurements. The measurement is safe and well tolerated by the patients and was accepted for its clinical use by Bioethical Committee of Medical University.

Ultrasound imaging

Fracture healing and its impairment have been identified by ultrasonography (US) and at an earlier stage than by radiography. [80, 81, 98-100]. Linear array transducers with frequencies ranging from 3,5 to 10+ MHz have been used in patients to evaluate bone healing. Transducer choice depends on the depth of bone being scanned. Penetration depth of the ultrasound is inversely correlated with frequency. A high frequency transducer will create an image with better resolution. Ultrasound imaging in fracture healing provides valuable information on callus quality [40, 68, 69, 82]. Criteria used for a healed fracture are based on echogenicity and the structure and surface of the fractured bone. Callus formation is ultrasonographically assessed using several criteria namely: haematoma organization, bone edges resorption, the echogenic density of tissue bridging the fracture gap, callus mineralization, and continuity of the bone surface. Intramedullary nail or internal callus may be identified until bone surface continuity is restored.

Six distinct time correlated US stages of bone healing have been reported.

Sharp, well defined bony edges are seen US in the first stage (7–11 days). Hypoechoic hematoma lies superficial to, and in the fracture gap. In the 2nd stage (10–16 days), the edges of the fracture gap become less sharply delineated. On either side of the fracture gap periosteal reaction becomes visible and creates so-called ‘‘collars’’ [40, 68, 69, 98-100]. Twenty days after fracture the image may show mixed echogenic tissue that begins to calcify. This, 3rd stage ranges from 20 to 35 day after fracture [40, 50, 68, 69, 98-100]. Radiographs at this time still do not have evidence of healing or reaction [100]. The US image in the fracture gap becomes more homogeneous in the 4th stage (35–50 days), and the acoustic shadow returns because of progressive mineralization. Well developed callus is usually greater than presented on the radiogram. Callus became progressively more hyperechoic with its structure changing from homogeneous (fresh hematoma) to inhomogeneous, and then to homogeneous again with mature callus formation. Radiographs at this time have signs of a calcifying periosteal callus. The callus begins to remodel in the 5th stage (50–90 days). The high difference in acoustic impedance between mineralized tissue and soft tissue reappear that may lead to reflection artifacts. In the latest stage (90–140 days) remodeling continues. Longitudinally oriented reverberation artifacts appear caused by the difference in acoustic impedance between bony surface and soft tissues. Their orientations parallel to the bony surface. The amount of callus varies considerably with age, the stability of the fixation, and fracture location. The amount of callus visible on radiographs depends on its mineralization and maturation (cancellous into lamellar bone). Ultrasound provides useful and detailed view of fracture healing but it should be used as a complementary method together with radiogram. The disadvantage of ultrasonographic evaluation of the fracture healing, is the fact that US is considered to be operator dependent. The physician performing ultrasonographic evaluation should remember that the probe evaluates a small section of bone at a time and longitudinal scan is necessary to avoid missing part of the fracture line. Ultrasound image can be used to document complete fracture healing earlier than conventional radiogram in long bone fractures.

Radiology

The regenerating bone, newly formed bone and achievement of union during fracture healing usually are assessed by observing standard plain radiographs. Direct evaluation of the radiogram by orthopaedic surgeon or skeletal radiologist for fracture healing assessment and prognosis is most frequently used. Dating of fracture healing on the basis of radiographic appearance is not often reported in the literature. Islam et al. [55] established a timetable for expected radiographic changes visible during bone healing in otherwise healthy children. Most of the articles on fracture repair and dating are derived from animal studies focusing on histological changes. Hammer et al. [49] have shown that the probability of correct radiological evaluation of the stage of union in tibial fractures is only about 50%. Panjabi et al. [95] measured different predictive variables on plain radiography in a standardized fracture model in rabbits and concluded the information gained from standard radiographs was not sufficient to accurately predict the strength of a healing fracture. Oblique radiographs are thought to be helpful [29].

Wade and Richardson [122] suggested that clinical or radiological methods are inaccurate, but together provide a good indicator in clinical practice when a fracture has healed. Young et al. [125] evidenced that radiographs are relatively insensitive and cannot predict the mechanical properties of the regenerate bone quantitatively. The introduction of digital radiography improves quantifying radiographs [32]. A noninvasive method to assess the fracture healing may utilize digital radiography [57] as well plain classic X-ray. The investigations were performed on a series of consecutively collected radiographs of patients prospectively observed in out-patient fracture clinic. Images were digitalized using steady positioned 5 Mpixel digital camera. This method was sensitive in assessing visit to visit healing progress. The high correlation between the optical density average value and the clinical assessment was evidenced. An aluminium step wedge placement on selected images and a region of interest outline around the fracture callus is necessary for further quantitative analysis. Methods enhancing radiological evaluation enormously improve reliability of the quantitative assessment. Tiedeman et al. [115] photometrically assessed the bone density and found a strong correlation ($r2 = 0.79$) between the intensity of light transmitted through standard radiographs and bending rigidity using a fracture model in dogs. Several features of digital radiography suggest its use in predicting the status in fracture healing: the improved detector efficiency, the wide exposure latitude, and the capabilities of digital processing [77]. Digital radiography allows for early assessment of the newly formed callus. In addition, the zoom image capability of the workstation can be used to examine radiographs under higher magnification.

Radiographs of fracture cases are assessed usually by orthopaedic surgeon or skeletal radiologist. Following radiographic determinants of fracture healing are commonly used:

- the presence of sharp or blurred fracture margins;
- loss of distinct fracture line;
- a gap between fracture fragments (measured in millimetres) in actual X-ray and compared with earlier radiographs to determine changes in spacing;
- bone density (sclerotic fracture margins);
- periosteal reaction and its subsequent incorporation into the cortex;
- presence of callus (w/ IM nail or external fixator);
- callus density (compared with that of adjacent cortex);
- bridging (partial or whole) at the fracture site;
- remodeling, determined by a loss of a focal cortical bump or an increase in the obtuse angle of new bone with cortex.
- restoration of cortical continuity.

In conservatively treated cases with cast the accuracy of detecting particular radiographic features decreases. Fracture margins are blurred on almost 60% of radiographs obtained 1 week after injury. Periosteal reaction is rather not observed on any radiograph obtained before 2 weeks but it is evident 4 weeks after injury. After 7 weeks, periosteal new bone seems to be separable from cortex on only 10% of radiographs, and remains separable in 11% 13 weeks after injury. Periosteal incorporation in cortex is not visible after 3 weeks and is present in almost 60% after 10 weeks. Fracture gap widens 2 weeks after injury in 10% of the cases. It is noted in more than 50% of radiographs 4 and 6 weeks after fracture, but is rarely seen after 7 weeks. The gap size decrease after 8 or more weeks after injury in more than 90% of fractures. Sclerotic edges at the fracture margins rarely appear 2 weeks after injury. Highest density at the fracture margins is observed in most of the fracture cases (85%) from 4 to 6 weeks after fracture.

An increase in bone density compared with that of adjacent bone at the fracture margin is not visible any more after 11 weeks. Callus calcification was not evidenced before 2 weeks after injury. It is well seen 4 weeks after injury. In 50% of fractures between 8 and 10 weeks after injury callus density is lower than that of adjacent cortex. Callus density steady rise after 10 weeks and achieve a density equal to or greater than that of cortex in 90% of cases.

Three weeks after injury partial bridging may be noted. Bridging is evidenced in 50% of fractures at 8 weeks. The full bridge of fracture after 10 weeks in 40% (disappearance of fracture line). Remodelling may not be observed earlier than 4 weeks after injury, but evidence of remodelling after 8 weeks is obvious.

Computer tomography

Computed tomography (CT) provides a 3-dimensional dataset of X-ray attenuation coefficients expressed in Hounsfield’s units. Computer Tomography shows some advantages over conventional X-rays in evaluation of early fracture healing. It may provide additional information in selected cases [15, 26, 35, 107].

Braunstein et al. [15] have found that Computed Tomography may be of value in the evaluation of fractures of long bones in those cases in which clinical examination and plain radiographs fail to give adequate information as to the status of healing. A little overall accuracy of the radiologic fracture healing measurement is added by CT scan evaluation [107] and isotope scans [112].

The presence of metallic implants is a limiting factor. Metabolic activity cannot be measured, and no functional imaging can be performed with CT. Methods of fracture healing assessment described by Grigorian et al. [47] are reliable tools that are able to detect early changes in normal bone healing and may serve as useful additions to subjective image analysis in monitoring fracture healing in clinical trials.

Quantitative evaluation showed good intraobserver and interobserver reproducibility and a statistically significant correlation to qualitative changes.

Grigoryan et al. [47] compared qualitative assessment of conventional radiography to quantitative and qualitative features of fracture healing using volumetric computed tomography (CT). Quantitative assessment of CT density changes (Hounsfield units [HU]) in the fracture gap. They used MEDx multimodality image analysis software to perform analysis by drawing freeform regions of interest (ROI) covering the fracture gap on baseline (week 1) images and by automated registration of the follow-up images to the baseline co-ordinate system.

Blurring of fracture margins, similarly to radiographic findings, remains the earliest observed sign of healing that appears almost 3 weeks after injury on CT images.

Earlier detection of evident external callus formation is possible with CT technique. Compared with conventional X-rays, CT images allow for more complete and detailed visualization of healing, often obscured by cast or fixation hardware superimposition.

Sclerotic fracture lines develop and subsequently disappear. The timing for this sign varies due to different fracture type. By CT images the fracture lines progressed to sclerotic by week 4 in radial fractures and 12 in tibial. Fracture margins sclerotic sign became invisible by week 12 in radial fracture and in tibial shaft fracture disappeared by week 16. External Callus formation of the periosteal reactive bone appears as early as 4 weeks postfracture with mean time of the first appearance approximately 7 weeks on CT scan.

CT image intensity allows performing quantitative evaluation.

In daily practice CT may be used for cases to ensure the treating physician of the degree of healing based on clinical exam and the appearance on conventional radiographs.

Computer Tomography may be sensitive enough to be used in clinical research involving the early stages of fracture healing and to evaluate more complex issues such as occult fractures, soft tissue injury, and fixation hardware–bone interaction. CT with volumetric reconstruction appears to offer the best balance between convenience, cost, availability, and ability to quantify calcified tissue changes. Own experience is based on 57 patients, 29 women and 28 men, with 61 long bone fractures examined to determine the quality of bone union using spiral Computer Tomography (GE Pro Speed SX, Slice width 2-3 mm, table shift 3-4 mm, image reconstruction 1,5 mm, 120 kV, 100 mAs). Patients were examined averagely 56 weeks after fracture. Multiplanar and volumetric reconstructions determined fracture healing impairment in all except one patient (28 delayed unions and 32 nonunions). Late Computer Tomography exam allows to determine fracture fragments location, and fracture gap size and finally to confirm delayed union. However, some doubts remain while formulating definitive CT diagnosis.

The CT results may be improved by quantitative exam procedure utilizing peripheral QCT. Calibration compares examined bone and phantom containing known concentration of bone mineral equivalent [104, 105]. All the different modalities were used to predict the mechanical stability of the healing bones.

DEXA

Dual energy x-ray absorptiometry (DEXA) is the most accurate technology available for measurement of bone mass in vivo. It mainly is used for the diagnosis of osteoporosis. Densitometric methods were used to demonstrate how the period of nonweightbearing after a bone injury influences the amount of bone loss [41, 121]. Further densitometric evaluation of long bones was employed to establish the stage o fracture healing. With DEXA, bone mineral density and bone mineral content can be measured as parameters of the bone density and bone mass, respectively. During the process of bone healing, mineralization of the callus leads to higher bone density and bone mass of the callus tissue. Therefore, DEXA could be useful in evaluating bone healing. A good correlation between mechanical properties and DEXA measurements has been described in a in a well-defined osteotomy model, [71, 73, 74, 78] and DEXA already has been shown to be a good predictor of atrophic nonunion in a comparable model. The fractured bone and the contralateral should be scanned simultaneously to assess DEXA Scans. Different regions of interest, bone mineral density and bone mineral content need to be measured. The values of bone mineral density and bone mineral content of the fractured bone are expressed as a percentage of the contralateral, intact tibia. Therefore, DEXA measurements possibly could be used as parameters for evaluation of fracture healing. An increase in mineralization of the callus, as measured with DEXA, leads to an increase in strength of a healing fracture [10]. There are some limitations of DEXA in evaluation of fracture healing due to examination technique and procedures. Prospective clinical studies are required to compare DEXA with other assessment methods including radiography, PET, CT and others and provide more insight into the role of DEXA in the assessment of fracture healing. Furthermore, the detection of hypertrophic nonunions might be difficult with DEXA. Abundant callus formation that occurs in hypertrophic nonunions could give a false positive outcome of DEXA measurements. The presence of metallic implants may produce the consequent artifacts. Markel and Bogdanske [72] reported that presence of metallic fracture fixation devices caused significant increase in the precision error, compared with measurements without fixation devices. The new software implemented to DEXA devices will probably correct the artifacts caused by metallic implants and would significantly improve the evaluation of fracture healing.

Fracture Healing Module of Relative Optical Density Image Analysis System

Direct evaluation of the radiogram for fracture healing assessment and prognosis is most frequently used. However, the radiogram may provide more information than experienced radiologist's eye is able to see. Computer aided diagnostic (CAD) methods operate on digitalized images. The analyses of the digitalized image were introduced for bone assessment, bone regeneration around implants, bone defects or erosions or fractures healing and union prediction [103, 111]. CAD methods are rarely implemented in orthopedics. Ning et al. [90] utilized the information from pairs of orthogonal images at different stages of healing to generate a 3D reconstruction of the callus which builds up during the treatment, thus facilitating understanding and enabling assessment of the process of fracture healing. They used three-dimensional (3D) reconstruction from two orthogonal images and a Monte Carlo program MCNP4B to simulate the x-ray images. Beltrame et al. [8] proposed a useful evaluation tool to a wide clinical scenario that introduces an innovative calibration algorithm for a semi-quantitative analysis of non-standardized digitized X-ray images. They selected and analyzed three time invariant regions (ROI) of radiographs for calibration on a unique standardization scale. The accuracy of the normalization method for X-ray films was successfully validated by using an aluminum step wedge for routine X-ray exposures as tool to standardize serial radiographs (Pearson correlation test: R(2) = 0.96). The abilities of this innovative image-processing algorithm were applied to investigate the progression of the new bone deposition within ceramic scaffolds used as osteoconductive substitute in large bone defects on a large animal model.

Computer assisted densitometric image analysis (CADIA) of bone density was the first developed computerized method to improve quantification of bone density on radiographs with or without previous standardization [14, 16, 21, 53, 113, 116, 109]. Standardization covers not only geometry but image conversion to unified optical density units namely Al equivalents. CADIA allowed performing an assessment on geometrically standardized images converted in Al equivalents. Katanec et al. [56] developed the new CADIA software to quantify bone density on dental radiographs without previous standardization. They digitized radiographs with a high resolution CCD camera and performed densitometric analysis in 10 points at the edge of the bone defect. More fine observations were done by Fourmousis et al. [33]. They assessed radiographically the effects of scaling/root planning combined with antibiotic therapy using tetracycline fibers (TCF): (I) on alveolar bone density and linear descriptors and (II) on supracrestal soft tissue density. Standardized periapical radiographs served to monitor mean changes in the linear parameters and changes in density (CADIA) observed at multiple sites.

Standardized methods require all radiographs processed with the same exposure parameters set at kVp, mA, and seconds at an ISO cm focus-to-film distance and also automatic film processor. Image capture and digitization have improved recently. Methods used for digitization and image capture have changed from flatbed scanner equipped with transparency adapter, dedicated radiographic scanners through CCD with frame grabbers, digital cameras to direct utilization of digital radiography [20, 60,114].

The later is becoming a golden standard for paperless and filmless radiography. The captured image of the radiograph is feasible to display, archive and analyse on the computer.

The range of optical densities in the radiographic image was converted into 256 gray scale values, with zero representing the black areas on the image and 256 representing the lightest area on the image. Each digitized radiogram becomes the baseline image for itself. All image capture and analysis were performed with a personal computer and 1024 x 768 resolution display. All algorithms used for the Fracture Healing Module are part of a software package (RODIA System developed for the Radiographic Assessment Enhancement) [45, 58]. All digitized images were saved on hard disk and copied on CD ROMs. The principles for algorithms were elaborated to enhance digital or digitalized radiographic image analysis. The Region of Interest around the fracture gap is determined. Widths, optical density of the fracture gap as well adjacent bone to fracture line constitute the features to compare in time. The optical density matrix is computed from digital image based on x and z co-ordinates of each point of the image. The z co-ordinate represents optical density value. 3D contour map plot for x, y and z show the bone fragment transformed into the pseudo 3-dimentional diagram assuming a surface of the solid figure available for multidirectional evaluation. An image transformation and further analysis determine fracture healing rate expressed as a per cent of the optical density value of fracture gap compared to adjacent intact bone calculated for each consecutive image.

Every examination consists of:

- input of optical density value (ODV) from ROI
- indication of approximate end points of fracture gap
- calculation of fracture gap average ODV
- calculation of area under achieved curve on the graph (2 D) based on ODV values
- comparison of the results
- results graphic visualisation, calculations and predictions.

The method precisely quantitates the result and allows detecting early steady values of optical density within fracture gap denoting delayed union. An application requires approximate localization of examined region with fairly exact image scale, rotation, etc) and image calibration and restoring system. 3D plot improves the gap shape observation and changes of its shape during fracture healing. Multiple locations probing of healing values across the fracture gap lead to reliable final healing results.

Clinical cases based on series of radiographic follow-up of the fracture patients present utility of the RODIA System and its successful application. Fracture surgeon achieves then healing rate curve and approximate union time prediction that may influence managing the patient.

Presented method of the digital image analysis together with easy image capture can be easily applied in daily clinical practice by the busy orthopedic trauma surgeon and potentially feasible for telemedical consultation [44].

Originally developed RODIA system is a set of methods supporting radiologist or non-radiologist opinion. Skeletal radiology may utilize the application for evaluation of fracture healing or general or localized relative bone density changes. These reliable tools are able to monitor changes in normal bone healing and may serve as useful objective method in clinical trials. Quantitative evaluations that show good intraobserver and interobserver reproducibility are suitable for statistical analysis required for Evidence Based Medicine. Presented set of methods is modern, unique and inexpensive approach to classic radiology.

„3D Reconstructor”

An original software “3D Reconstructor” was developed based on three-dimensional reconstruction and measurements of various anatomical structures using DICOM files.

The loaded images sequence is initially modified i.e.: secondary layers are generated or reorganized. 3D Reconstructor v.2.0 allows also 2D reconstruction in custom surface led through data block and 3D reconstruction as well. The application is suitable for long bone fracture healing with three-dimensional presentation of fracture callus.

3D Reconstructor developed as Computer aided Diagnostic Method quantitatively has the potential to analyze details of X-ray and CT images. Enhanced evaluation of original CT DICOM files seems to be more accurate over not armed evaluation of fracture healing in selected cases. Zylkowski’s “3D Reconstructor” application reads DICOM files as well other graphical formats. The application was primarily designed for viewing, measuring and three-dimensional reconstructing of anatomical structures from image medical data (particularly for CT and MRI in neurosurgery). Its modular construction allows adding other functions spreading its applications. The bone is selected from loaded CT-sequence data for each patient based on Hounsfield units (density), starting from reference point set by user. It allows dividing the selection into sub-models by voxel’s Hounsefield’s Unit values and user defined threshold-table. The sub-models were transformed next to three-dimensional triangular meshes using implemented Marching Cubes Algorithm. The color and transparency is set manually or automatically for each mesh. Three-dimensional model utilizes OpenGL where the user may switch on/off lights and transparency. Ortho and perspective presentations permit to perform basic measurements (distance, angle, volume).

Transparent model of bone and callus are visualized through sub models with bone (above 500HU) and callus preset values (200-500HU). Surface topograms are directed along the fracture gap with transparent isosurfaces preset at various levels of Hounsfield Units.

Fracture gap detection enhancement is made also on topograms showing rotation of the measured surface around its center for Y axis goes through fracture gap. Additionally, 2 D pseudo X-ray presentation can be reconstructed from CT DICOM file. Internal view from inside of the callus enhances 3D reconstruction for evaluation of fracture healing and bone union impairment.

In some clinical cases CAD quantitative assessment of the fracture gap and callus was successfully performed using 3D Reconstructor software. Higher accuracy of pseudoarthrosis and delayed union diagnosis was achieved utilizing “3D Reconstructor”. Computer Aided Diagnostic Methods may improve quantitative analyzing of X-ray and CT images. Still ongoing project, “3D Reconstructor” seems to be more accurate over not “armed” evaluation of original CT DICOM files of fracture healing in selected cases.

Other methods

Various methods had been described for fracture healing quantitative evaluation including: strain gauge instrumentation of fixation devices [27, 94], auscultatory percussion [64, 79], vibrational analysis [24, 31, 89], acoustic emission [54, 124], resonant methods [11, 22, 23, 92, 102, 110], impulse response [86, 87], spectral analysis [28] and scintigraphy [7, 112, 123]. Bone scintigraphy is based on metabolic activity, and therefore could be used for evaluation of bone healing.

However, it has been shown that fracture healing cannot be quantified reliably with bone scintigraphy [123]. Using scintigraphy in the determination of viability of bone grafts has not been successful either [9, 46]. Benign orthopedic conditions may benefit from use of bone positron emission tomography [78] a new imaging method using labeled fluorine (18F) as a tracer to measure bone metabolic activity. Although some research has been done to evaluate fracture healing [5] no validation for using positron emission tomography in fracture healing has been established. Moreover, positron emission tomography is an expensive imaging method and it is not available in many departments.

Methods that usually address direct or indirect stiffness measurements or other features of fracture callus [25, 118]. Assessment of the bone by steady state analysis utilizes bone response to forced harmonic vibration that relies on natural frequencies which in turn are a function of stiffness and mass [4, 25].

Mechanical testing is able to measure stiffness pattern of healing in indirect and direct way [6, 19, 85, 95, 101] with either invasive or noninvasive procedures. Fractures treated with an external fixator were evaluated using strain gauges on the external fixator [85, 93], however the accuracy of measurement decreases with pin loosening. A measurement of direct stiffness is typically measured after 6–8 weeks. The tibial fractures are often the target for evaluation.

Measurement may also be performed by angulation at the fracture site quantifying clinical assessment of bone union utilizing X-rays [49, 122], or surface measurements after using four point bending. If the value of the stiffness reached 7 Nm/degree (°) no fractures failed to heal after 20 weeks. The radiogram can show some evidence of bone healing indicated by calcification at the callus and fracture gap [47, 49].

Accurate measurement of angulation is available for external fixation but is not applicable to internally fixed fractures. The stiffness value of 15 Nm/° of tibial fractures in the anterior–posterior plane in patients treated with external fixators denotes healing. The method is simple to use in the clinical setting and accurate (total error of 3%).

Methods described above in details including radiography, Computed Tomography, Ultrasound imaging, Ultrasonometry and Computer Aided Diagnosis (RODIA, 3D Reconstructor) utilizing X-ray and CT data were clinically validated and tested.

Despite of large utility of those methods in experimental studies their practical application remains limited.

Conclusions

Subjective physical examination of bone union may not lead to correct conclusions, so an assessment using a more objective method of analyzing the degree of healing of a fracture is required for the identification of the completion of fracture healing, and treatment discontinuation. In general, histology is considered an excellent evaluation method however as a direct but invasive method it may not be applicable in most clinical cases.

The quantitative measurement is required for fracture healing evaluation. Having definite measurements an orthopaedic trauma surgeon would be able to predict refractures, monitor healing processes, and set an exact date for the end of immobilization or fixation removal. Realistic and quantitative evaluation of fracture healing may create a new era for orthopaedic trauma science. The analysis of various factors, including biological, pharmacological, physical, mechanical, genetic and others, influence on fracture healing remains great challenge in orthopaedic medicine.

Many non-invasive quantitative techniques for measuring fracture healing have been reported over the past decades. Their acceptance is not high and regular use is rare.

A reliable method should allow the determination time of adequate union by its strength, quality and quantity, but the busy orthopaedic surgeon require suitable methods with user friendly interface to implement them into daily clinical practice.

BIBLIOGRAPHY

[1] Abendschein W, Hayatt GW. Ultrasonics and physical properties of healing bone. J Trauma 12:297–301, 1972
[2] Anast GT, Fields T, Siegel IM. Ultrasonic technique for the evaluation of bone fractures. Am J Phys Med. 37(3):157-159, 1958
[3] Andreev VN, Emelianov VA, Kuzmin VM. Acoustic fracture indicator Ortop Travmatol Protez. (Rus.) (8):69-70, 1980
[4] Aranzulla PJ, Muckle DS, Cunningham JL. A portable monitoring system for measuring weight-bearing during tibial fracture healing. Med Eng Phys. 20(7), 543-548, 1998
[5] Ashcroft GP, Evans NT, Roeda D, Dodd M, Mallard JR, Porter RW, Smith FW. Measurement of blood flow in tibial fracture patients using positron emission tomography. J Bone Joint Surg 74B:673-677,1992
[6] Ashman R, Rho J, Turner C. Anatomical variation of orthotropic elastic moduli of the proximal human tibia. J Biomech 22, 895–900, 1989
[7] Barros JW, Barbieri CH, Fernandes CD. Scintigraphic evaluation of tibial shaft fracture healing. Injury. 31(1), 51-54, 2000
[8] Beltrame F, Cancedda R, Canesi B, Crovace A, Mastrogiacomo M, Quarto R, Scaglione S, Valastro C, Viti F. A simple non invasive computerized method for the assessment of bone repair within osteoconductive porous bioceramic grafts. Biotechnol Bioeng. 20;92(2):189-198, 2005
[9] Berggren A, Weiland AJ, Ostrup LT: Bone scintigraphy in evaluating the viability of composite bone grafts revascularized by microvascular anastomoses, conventional autogeneous bone grafts, and free non-revascularized periosteal grafts. J Bone Joint Surg 64A:799-809,1982
[10] Blokhuis T.J., den Boer F.C., Bramer J.A.M., van Lingen A., Roos J.C., Bakker F.C., Patka Peter, Haarman H.J. Evaluation of Strength of Healing Fractures With Dual Energy Xray Absorptiometry Clin Orthop Rel Res 260-268, 2000
[11] Borgwardt Christensen A, Tougaard L, Dyrbye C, Vibe-Hansen H. Resonance of the human tibia. Method, reproducibility and effect of transection. Acta Orthop Scand. 53(6), 867-874, 1982
[12] Bossy E, Talmant M, Laugier P. Effect of bone cortical thickness on velocity measurements using ultrasonic axial transmission: a 2D simulation study. J Acoust Soc Am. 112(1):297-307, 2002
[13] Bossy E, Talmant M, Peyrin F, Akrout L, Cloetens P, Laugier P. An in vitro study of the ultrasonic axial transmission technique at the radius: 1-MHz velocity measurements are sensitive to both mineralization and intracortical porosity. J Bone Miner Res. 19(9):1548-1556, 2004
[14] Bragger U, Hugel-Pisoni C, Burgin W, Buser D, Lang NP. Correlations between radiographic, clinical and mobility parameters after loading of oral implants with fixed partial dentures. A 2-year longitudinal study. Clin Oral Implants Res.7(3):230-239, 1996
[15] Braunstein EM, Goldstein SA, Ku J, Smith P, Matthews LS. Computed tomography and plain radiography in experimental fracture healing. Skeletal Radiol. 15:27-31,1986
[16] Brown GD, Mealey BL, Nummikoski PV, Bifano SL, Waldrop TC. Hydroxyapatite cement implant for regeneration of periodontal osseous defects in humans. J Periodontol. 69(2):146-157, 1998
[17] Busse JW, Bhandari M, Kulkarni AV, Tunks E. The effect of low-intensity pulsed ultrasound therapy on time to fracture healing: a metaanalysis. CMAJ, 19; 166(4):437–441, 2002
[18] Camus E, Talmant M, Berger G, Laugier P. Analysis of the axial transmission technique for the assessment of skeletal status. J Acoust Soc Am. 108(6):3058-3065, 2000
[19] Chehade MJ, Pohl AP, Pearcy MJ, Nawana N. Clinical implications of stiffness and strength changes in fracture healing. J Bone Joint Surg Br. 79(1), 9-12, 1997
[20] Chen S.K., Hollender L. Digitizing of radiographs with a flatbed scanner J Dentistry 23; 4; 205-208, 1995
[21] Cochran DL, Nummikoski PV, Higginbottom FL, Hermann JS, Makins SR, Buser D. Evaluation of an endosseous titanium implant with a sandblasted and acid-etched surface in the canine mandible: radiographic results. Clin Oral Implants Res. 7(3):240-252, 1996
[22] Collier RJ, Donarski RJ. Non-invasive method of measuring resonant frequency of a human tibia in vivo. Part 1. J Biomed Eng. 9(4), 321-328, 1987

[23] Collier RJ, Donarski RJ. Non-invasive method of measuring the resonant frequency of a human tibia in vivo. Part 2. J Biomed Eng. 9(4), 329-331, 1987
[24] Cornelissen M, Cornelissen P, van der Perre G, Christensen AB, Ammitzboll F, Dyrbye C. Assessment of tibial stiffness by vibration testing in situ: III. Sensitivity of different modes and interpretation of vibration measurements. J Biomech 20, 333–342, 1987
[25] Cunningham JL, Kenwright J, Kershaw CJ. Biomechanical measurement of fracture healing. J Med Eng Technol.14 (3):92-101, 1990
[26] den Boer FC, Bramer JAM, Patka P, et al: Quantification of fracture healing with three-dimensional computed tomography. Arch Orthop Trauma Surg 117:345-350,1998
[27] Deszczynski J. New concepts of monitoring and assessment of bone union stiffness in fractures treated by Dynastab DK external fixation Chir Narzadow Ruchu Ortop Pol. (pol.) 64(6), 649-656, 1999
[28] Doemland HH, Jacobs RR, Spence J, Roberts FG. Assessment of fracture healing by spectral analysis. J Med Eng Technol. 10(4), 180-187, 1986
[29] Ebraheim NA, Savolaine ER, Patel A, et al: Assessment of tibial fracture union by 35-45 degrees internal oblique radiographs. J Orthop Trauma 5:349-350,1991
[30] Einhorn TA. Enhancement of fracture-healing. J Bone Joint Surg Am 77(6):940–956, 1995
[31] Fellinger M, Leitgeb N, Szyszkowitz R, Peicha G, Passler J, Seggl W, Schanner A. Early detection of delayed union in lower leg fractures using computerised analysis of mechanical vibration reactions of bone for assessing the state of fracture healing. Acta Orthop Trauma Surg 113, 93–96, 1994
[32] Floyd Jr CE, Chotas HG, Dobbins III JT, Ravin CE: Quantitative radiographic imaging using a photostimulable phosphor system. Med Phys 17:454-459, 1990
[33] Fourmousis I, Tonetti MS, Mombelli A, Lehmann B, Lang NP, Bragger U. Evaluation of tetracycline fiber therapy with digital image analysis. J Clin Periodontol. 25(9):737-745, 1998
[34] Gerlanc M, Haddad D, Hyatt GW, Langloh JT, St Hilaire P. Ultrasonic study of normal and fractured bone. Clin Orthop Relat Res. (111):175-80, 1975
[35] Gershuni DH, Skyhar MJ, Thompson B, Resnick D, Donald G, Akeson WH. A comparison of conventional radiography and computed tomography in the evaluation of spiral fractures of the tibia. J Bone Joint Surg Am. 67(9):1388-95, 1985
[36] Gill PJ, Kernohan G, Mawhinney IN, Mollan RA, McIlhagger R. Investigation of the mechanical properties of bone using ultrasound. Proc Inst Mech Eng [H] 203(1):61–63, 1989
[37] Glinkowski W., Jędral T ., Kornacki M. , Latuszek A. Ultrasonic methods for compact bone of long bones evaluation in experimental and clinical settings Acta BioOpt.Inform.Med. (pol.) 3, 2-4, 141-143, 1997
[38] Glinkowski W., Jędral T ., Kornacki M. , Latuszek A. Evaluation method of long bones compact tissue for parametric assessment of fracture healing in preliminary clinical studies Med Sport (pol.) 13, 11, 23-26, 1997
[39] Glinkowski W., Jędral T ., Kornacki M. , Latuszek A. Metod of Ultrasonometric Fractures Healing Evaluation Of Long Bones Bone 23, 5 (Suppl), S544. (Abstract SA 069), 1998
[40] Glinkowski W. Fracture healing of long bones monitoring with ultrasonography Ortopedia Traumatologia i Rehabilitacja (Pol.) 3, 2000
[41] Glinkowski W. Bone loss due to extremity immobilization after injury Med Sport (pol.) 9; 21-22, 1997
[42] Glinkowski W., Czajkowska A., Jędral T. Ultrasonometry of compact bone of tibia. Preliminary report. Med Sci Monit 4, (Suppl.2), 125-127, 1998
[43] Glinkowski W., Górecki A. Clinical experiences with ultrasonometric measurement of fracture healing Technology and Health Care 13, 1–13, 2006
[44] Glinkowski W., Kornacki M. Telemedicine – New concept of the medical care Acta Bio-Optica et Informatica Medica (pol.) 2; 3-4; 155-156, 1997
[45] Glinkowski W., M.Kornacki, M.Ambroziak, A.Górecki Relative Optical Density Image Analysis (RODIA©) of digitized radiograms for tibial fracture healing monitoring International Congress Series 1268, 1292, 2004
[46] Greenberg BM, Jupiter JB, McKusick K, May Jr JW: Correlation of postoperative bone scintigraphy with healing of vascularized fibula transfer: A clinical study. Ann Plast Surg 23:147-154,1989
[47] Grigoryan M., Lynch J. A., Fierlinger A. L., Guermazi A, Fan B., MacLean D.B., MacLean A., Genant H.K. Quantitative and Qualitative Assessment of Closed Fracture Healing Using Computed Tomography and Conventional Radiography Acad Radiol 2003; 10:1267–1273
[48] Hadjiargyrou M, McLeod K, Ryaby JP, Rubin C. Enhancement of fracture healing by low intensity ultrasound. Clin Orthop 355(Suppl):S216–229, 1998
[49] Hammer R, Hammerby S, Lindholm B. Accuracy of radiological assessment of tibial shaft fractures in humans. Clin Orth RR 199:233–238, 1985

[50] Hannesschlager G, Reschauer R: Sonographic follow-up of secondary fracture healing. Initial experiences with morphologic and semiquantitative assessment of periosteal callus formation. Rofo Fortschr Geb Rontgenstr Neuen Bildgeb Verfahr 153:113–119, 1990
[51] Hawkins RA. Bone metabolic activity measured with positron emission tomography and [18F] fluoride ion in renal osteodystrophy: Correlation with bone histomorphometry. J Clin Endocrinol Metab 77:949-955,1993
[52] Heckman JD, Ryaby JP, McCabe J, Frey JJ, Kilcoyne RF. Acceleration of tibial fracture healing by non-invasive, low-intensity pulsed ultrasound. J Bone Joint Surg Am 76(1):26–34, 1994
[53] Hermann JS, Cochran DL, Nummikoski PV, Buser D. Crestal bone changes around titanium implants. A radiographic evaluation of unloaded nonsubmerged and submerged implants in the canine mandible. J Periodontol. 68(11):1117-1130, 1997
[54] Hirasawa Y, Takai S, Kim WC, Takenaka N, Yoshino N, Watanabe Y. Biomechanical monitoring of healing bone based on acoustic emission technology. Clin Orthop Relat Res. 402, 236-244, 2002
[55] Islam O., Soboleski D., Symons S., Davidson L. K., Ashworth M. A., Babyn P.Development and Duration of Radiographic Signs of Bone Healing in Children AJR 175:75-78, 2000
[56] Katanec D, Kobler P, Slaj M, Anic I, Milosak T. Computer assisted densitometric image analysis (CADIA) of bone density in periradicular bone defects healing. Coll Antropol.22 Suppl:7-13, 1998
[57] Kolbeck S., Bail H., Weiler A., Windhagen H., Haas N., Raschke M. Digital Radiography A Predictor of Regenerate Bone Stiffness in Distraction Osteogenesis Clinical Orthopaedics And Related Research 366:221-228, 1999
[58] Kornacki M., Glinkowski W. Relative Optical Density Image Analysis (RODIA). Clinical application – preliminary report of FHM and IEE subsystems usage Med Sci Monit 4 (Suppl.2), 136-139, 1998
[59] Kristiansen TK, Ryaby JP, McCabe J, Frey JJ, Roe LR. Accelerated healing of distal radial fractures with the use of specific, low-intensity ultrasound. A multicenter, prospective, randomized, double-blind, placebo-controlled study. J Bone Joint Surg Am 79(7):961–973, 1997
[60] Krupinski E, Gonzales M, Gonzales C, Weinstein RS. Evaluation of a digital camera for acquiring radiographic images for telemedicine applications. Telemed J E Health. 6(3):297-302, 2000
[61] Lee KI, Yoon SW. Feasibility of bone assessment with leaky Lamb waves in bone phantoms and a bovine tibia. J Acoust Soc Am. 115(6):3210-3217, 2004
[62] Lefebvre F, Deblock Y, Campistron P, Ahite D, Fabre JJ. Development of a new ultrasonic technique for bone and biomaterials in vitro characterization. J Biomed Mater Res. 63(4):441-446, 2002
[63] Lieberman JR, Daluiski A, Einhorn TA. The role of growth factors in therepair of bone. Biology and clinical applications. J Bone Joint Surg Am 84-A(6):1032–1044, 2002
[64] Lipmann RK. The use of auscultatory percussion for the examination of fractures. J Bone Joint Surg 14, 118, 1932
[65] Lorenc S., Glinkowski W., Rydzyński P., Lorenc R. The influence of the height of body mass on ultrasound measurements of bones in healthy Polish women Pol Merkuriusz Lek. 5(28):215-217, 1998
[66] Lowet G, van der Perre G. In vivo and in vitro measurement of ultrasound velocity in cortical bone. Stud Health Technol Inform. 40:201-220, 1997
[67] Lowet G, van der Perre G. Ultrasound velocity measurement in long bones: measurement method and simulation of ultrasound wave propagation. J Biomech. 29(10):1255-1262, 1996
[68] Maffulli N,Thornton A: Ultrasonographic appearance of external callus in long-bone fractures. Injury 26:5–12, 1995
[69] Maffulli N: Ultrasound for detection of fracture healing. J Orthop Trauma 13:395–396, 1999
[70] Malizos KN., Papachristos A.A., Protopappas V.C., Fotiadis D.I. Transosseous application of low-intensity ultrasound for the enhancement and monitoring of fracture healing process in a sheep osteotomy model Bone 38, 530–539, 2006
[71] Markel MD, Bogdanske JJ, Xiang Z, Klohnen A: Atrophic nonunion can be predicted with dual energy x-ray absorptiometry in a canine ostectomy model. J Orthop Res 13:869-875,1995
[72] Markel MD, Bogdanske JJ: Dual-energy X-ray absorptiometry of canine femurs with and without fracture fixation devices. Am J Vet Res 55:862-866,1994
[73] Markel MD, Chao EY: Noninvasive monitoring techniques for quantitative description of callus mineral content and mechanical properties. Clin Orthop 293:37-45,1993
[74] Markel MD, Wikenheiser MA, Morin RL, et al: Quantification of bone healing. Comparison of QCT, SPA, MRI, and DEXA in dog osteotomies. Acta Orthop Scand 61:487-498,1990
[75] Maylia E, Nokes LD.The use of ultrasonics in orthopaedics-a review. Technol Health Care. 7(1):1-28, 1999
[76] McKinley DW, Chambliss ML. Follow-up radiographs to detect callus formation after fractures. Arch Fam Med 9(4):373–374, 2000

[77] Meritt CRB, Tutton RH, Bell KA, et al: Clinical application of digital radiography: Computed radiographic imaging. RadioGraphics 5:397-414, 1985
[78] Messa C, Goodman WG, Hoh CK, Choi Y, Nissenson AR, Salusky IB, Phelps ME. Bone metabolic activity measured with positron emission tomography and [18F] fluoride ion in renal osteodystrophy: Correlation with bone histomorphometry. J Clin Endocrinol Metab 77:949-955,1993
[79] Misurya RK, Khare A, Mallick A, Sural A, Vishwakarma GK. Use of tuning fork in diagnostic auscultation of fractures. Injury. 18(1), 63-64, 1987
[80] Moed BR, Kim EC, van Holsbeeck M, Schaffler MB, Subramanian S, Bouffard JA., Craig JG. Ultrasound for the early diagnosis of tibial fracture healing after static interlocked nailing without reaming: histologic correlation using a canine model. J Orthop Trauma 12:200–205, 1998
[81] Moed BR, Subramanian S, van Holsbeeck M, Watson JT, Cramer KE, Karges DE. Craig JG, Bouffard JA. Ultrasound for the early diagnosis of tibial fracture healing after static interlocked nailing without reaming: clinical results. J Orthop Trauma 12:206–213, 1998
[82] Moed BR, Watson JT, Goldschmidt P, van Holsbeeck M. Ultrasound for the early diagnosis of fracture healing after interlocking nailing of the tibia without reaming. Clin Orthop Rel Res 310:137–144, 1995
[83] Moilanen P, Kilappa V, Nicholson PH, Timonen J, Cheng S. Thickness sensitivity of ultrasound velocity in long bone phantoms. Ultrasound Med Biol. 30(11):1517-1521, 2004
[84] Moilanen P, Nicholson PH, Karkkainen T, Wang Q, Timonen J, Cheng S. Assessment of the tibia using ultrasonic guided waves in pubertal girls. Osteoporos Int. 14(12):1020-1027, 2003
[85] Moorcroft CI, Ogrodnik PJ, Thomas PB, Wade RH. Mechanical properties of callus in human tibial fractures: a preliminary investigation. Clin Biomech (Bristol, Avon). 16(9), 776-782, 2001
[86] Nakatsuchi Y, Tsuchikane A, Nomura A. Assessment of fracture healing in the tibia using the impulse response method. J Orthop Trauma. 10(1), 50-62, 1996
[87] Nakatsuchi Y, Tsuchikane A, Nomura A. The vibrational mode of the tibia and assessment of bone union in experimental fracture healing using the impulse response method. Med Eng Phys 18(7), 575–583, 1996
[88] Nicholson PH, Moilanen P, Karkkainen T, Timonen J, Cheng S. Guided ultrasonic waves in long bones: modelling, experiment and in vivo application. Physiol Meas. 23(4):755-768, 2002
[89] Nikiforidis G, Bezerianos A, Dimarogonas A, Sutherland C. Monitoring of fracture healing by lateral and axial vibration analysis. J Biomech. 23(4), 323-330, 1990
[90] Ning J, McClean S, Cranley K. Using simulated annealing for 3D reconstruction of orthopedic fracture. Med Phys. 31(8):2184-2191, 2004
[91] Njeh CF, Hans D, Wu C, Kantorovich E, Sister M, Fuerst T, Genant HK. An in vitro investigation of the dependence on sample thickness of the speed of sound along the specimen. Med Eng Phys. 21(9):651-659, 1999
[92] Nokes LD, Mintowt-Czyz WJ, Fairclough JA, Mackie I, Howard C, Williams J. Natural frequency of fracture fragments in the assessment of tibial fracture healing. J Biomed Eng. 6(3), 227-229, 1984
[93] Ogrodnik PJ, Moorcroft CI, Thomas PB. A fracture movement monitoring system to aid in the assessment of fracture healing in humans. Proc Inst Mech Eng [H]. 215(4), 405-414, 2001
[94] Oni OO, Capper M, Soutis C. Monitoring fracture healing with strain gauges: the effect on strain values of strain gauge location on the pin circumference. Injury. 26(3), 181-182, 1995
[95] Panjabi MM, Walter SD, Karuda M, White AA, Lawson JP: Correlations of radiographic analysis of healing fractures with strength: A statistical analysis of experimental osteotomies. J Orthop Res 3:212-218, 1985
[96] Protopappas VC, Baga DA, Fotiadis DI, Likas AC, Papachristos AA, Malizos KN. An ultrasound wearable system for the monitoring and acceleration of fracture healing in long bones. IEEE Trans Biomed Eng. 52(9):1597-1608, 2005
[97] Protopappas VC, Fotiadis DI, Malizos KN. Guided ultrasound wave propagation in intact and healing long bones. Ultrasound Med Biol. 32(5):693-708, 2006
[98] Ricciardi L, Perissinotto A, Dabala M: External callus development on ultrasound and its mechanical correlation. Ital J Orthop Traumatol 18:223–229, 1992
[99] Ricciardi L, Perissinotto A, Dabala M: Mechanical monitoring of fracture healing using ultrasound imaging. Clin Orthop 71–76, 1993
[100] Ricciardi L, Perissinotto A, Visentin E: Ultrasonography in the evaluation of osteogenesis in fractures treated with Hoffman external fixation. Ital J Orthop Traumatol 12:185–189, 1986
[101] Richardson JB, Cunningham JL, Goodship AE, O'Connor BT, Kenwright J. Measuring stiffness can define healing of tibial fractures. J Bone Joint Surg Br 76(3), 389–394, 1994
[102] Roberts SG, Steele CR. Efficacy of monitoring long-bone fracture healing by measurement of either bone stiffness or resonant frequency: numerical simulation. J Orthop Res. 18(5), 691-697, 2000

[103] Rowiński J., Glinkowski W., Głębowski P. Estimation of optical density of bone tissue radiograms with laser densitometry In Proceedings of SPIE, Optics in Biophysics and Biochemistry, Editor -in - Chief M.Pluta, SPIE-PL; Vol.2, 143-148, 1996
[104] Ruegsegger P, Elssasser U, Anliker M, Gnehm H, Kind H, Prader H. Quantification of bone mineralization using computed tomography. Radiology 121:93–97, 1976
[105] Ruegsegger P, Kalender WA A phantom for standardization and quality control in peripheral bone measurements by PACT and DXA. Phys Med Bi 38:1963–1970, 1993
[106] Saulgozis J, Pontaga I, Lowet G, Van der Perre G. The effect of fracture and fracture fixation on ultrasonic velocity and attenuation Physiol Meas. 17(3):201-211, 1996
[107] Schnarkowski P, Redei J, Peterfy CG, et al: Tibial shaft fractures: Assessment of fracture healing with computed tomography. J Comput Assist Tomogr 19:777-781, 1995
[108] Schweighofer F, et al: Sonographische Frakturdiagnostik. Ultraschall 11:206–209, 1990;
[109] Sheaffer JC, Eleazer PD, Scheetz JP, Clark SJ, Farman AG. Endodontic measurement accuracy and perceived radiograph quality: effects of film speed and density. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 96(4):441-448, 2003
[110] Singh VR, Yadav S, Adya VP. Role of natural frequency of bone as a guide for detection of bone fracture healing. J Biomed Eng. 11(6), 457-461, 1989
[111] Słomczykowski M., Kmieciak M., Wolf L., Kołodziejczyk K. Bone radiogram digital assessment method Chir Narz Ruchu Ortop Pol (Pol.) 60; 3; 211- 215, 1995
[112] Smith M.A., Jones E.A., Strachan R.K., Nicholl J.J., Best J.J., Tothill P., Hughes S.P. Prediction of fracture healing in the tibia by quantitive radionucleotide imaging, J Bone Joint Surg 69B, 441–447, 1987
[113] Stentz WC, Mealey BL, Nummikoski PV, Gunsolley JC, Waldrop TC. Effects of guided bone regeneration around commercially pure titanium and hydroxyapatite-coated dental implants. I. Radiographic analysis. J Periodontol. 68(3):199-208, 1997
[114] Szot A, Jacobson FL, Munn S, Jazayeri D, Nardell E, Harrison D, Drosten R, Ohno-Machado L, Smeaton LM, Fraser HS. Diagnostic accuracy of chest X-rays acquired using a digital camera for low-cost teleradiology. Int J Med Inf. 73(1):65-73, 2004
[115] Tiedeman JJ, Lippiello L, Connolly JF, Strates BS Quantitative roentgenographic densitometry for assessing fracture healing. Clin Orthop 253:279-288, 1988
[116] Tillmanns HW, Hermann JS, Tiffee JC, Burgess AV, Meffert RM. Evaluation of three different dental implants in ligature-induced peri-implantitis in the beagle dog. Part II. Histology and microbiology. Int J Oral Maxillofac Implants. 13(1):59-68, 1998
[117] Tsuda-Futami E, Hans D, Njeh CF, Fuerst T, Fan B, Li J, He YQ, Genant HK. An evaluation of a new gel-coupled ultrasound device for the quantitative assessment of bone. Br J Radiol. 72(859):691-700, 1999
[118] Utvag SE, Reikeras O. Effects of nail rigidity on fracture healing. Strength and mineralisation in rat femoral bone. Arch Orthop Trauma Surg. 118, (1-2), 7-13, 1998
[119] Valentin-Opran A, Wozney J, Csimma C, Lilly L, Riedel GE. Clinical evaluation of recombinant human bone morphogenetic protein-2. Clin Orthop 2002; 395:110–120, 1998
[120] Van Der Perre G, Lowet G. In vivo assessment of bone mechanical properties by vibration and ultrasonic wave propagation analysis. Bone. 18 (1 Suppl): 29S-35S, 1996
[121] Van der Wiel HE, Lips P, Nauta J, et al: Loss of bone in the proximal part of the femur following unstable fractures of the leg. J Bone Joint Surg 76A:230-236,1994
[122] Wade R., Richardson J. Outcome in fracture healing: a review Injury, Int. J. Care Injured 32, 109–114, 2001
[123] Wallace AL, Strachan RK, Blane A, Best JJ, Hughes SP. Quantitative early phase scintigraphy in the prediction of healing of tibial fractures. Skeletal Radiol. 21(4), 241-245, 1992
[124] Watanabe Y, Takai S, Arai Y, Yoshino N, Hirasawa Y. Prediction of mechanical properties of healing fractures using acoustic emission. J Orthop Res. 19(4), 548-553, 2001
[125] Young JWR, Kostrubiak IS, Resnick CS, Paley D. Sonographic evaluation of bone production at the distraction site in Ilizarov limb-lenghthening procedures. Am J Roentgenol 154:125-128, 1990