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An alternative method in fixation of tibial transverse fractures by intramedullar nailing: Biomechanical and histopathologic investigation

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A new method was used in fixation of tibial bone fractures. Intramadular nailing (IMN) has been used into mid-diaphysis on left tibias of New Zeland rabbits (n = 5) via an *in vivo* work. To enable fixation of fracture, without causing too much screw damage on bone and avoiding malunion, nano- and micro-scale hydroxyapatite (HA) was coated at two ends (25 mm in length) of intramadular nails before implantation. After six weeks of survival period and sacrifizing, biomechanical tests and histopathologic examinations were executed. Such experiments have revealed that good stabilization and hence better fracture union for both treated IMN groups (NHA and MHA) over the standard IMN'. Pull-out tests showed the tensile strengths obtained to be significantly higher for the nano (NHA) and micro scale-MHA coated IMN compared to the uncoated standard IM nailing.

Key words: *bone fractures, intramedular nails, coating, fixation, biomechanics*

1. Introduction

Tibial bone fractures are pathology with the highest morbidity and mortality in its sub-extremity traumas. Treatment of severely comminuted supracondylar fractures of femurs and tibiaes continue to pose a challenge for the trauma surgeon, despite advancements in surgical technique and implant devices. Although most fractures are treated with conservative methods (splints, plaster detection, various bandages, etc.) surgical interventions are applied for substantial number of fractures [1]. The popularity of intramedullary nails (IMN) in the treatment of tibial object fractures increased continually, especially after the 1980's [2]. The healing of fracture is affected by various factors, but the stability is a significant factor in fracture union [3]. Intramedullar nailing is the gold standard treatment option for displaced closed or open tibial diaphyseal fractures [4]–[7] and the commonly accepted approaches to tibial nailing techniques [8].

Intramedullar nailing in the treatment of femur and tibial object fractures is, in recent times, very common because of advantages such as providing more joint mobility due to good stabilization, less soft tissue incision during implantation, only few angular and rotational deformities and high rate of healing fractures [9], [10]. However, Greitbauer et al. [13] have reported in a research that a burden applied at unengraved and self-locked intramedullary nailing was embarked on the self-locking screws, and that may cause some problems in implanting. Many methods exist in fixation of different bone fractures such as standard flexible nailing and static-dynamic self-

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locking intramedullar nails [7], [11]–[18]. Self-locking IMN' is the latest method applied, however, there are some difficulties in using areas like shoulder humereus that may cause impingement. Locking screws provide controlled dynamic compression effects but never shows static compression among fragments. It is also revealed in the literature that, beside the advantages of self-locking intramedullar nailing, the possible emergence of sceptic arthritis has some disadvantages like requiring the use of scopies, the surgeon is exposed to radiation, spoiling the gavages of the anterior cruciate ligament and the posterior cruciate ligament [19]. It was also reported that post-operational frontal knee pain may occur in patients who have undergone self-locking intramedullar nailing process, because of a tibia diaphysis fracture [18].

Hydroxyapatite (HA) is potentially enabled to be used for healing segmentary bone losses because it is an osteoconductive material in composing a good osteointegration with bone tissue [20]–[25]. Rothman et al. [27] have either reported in their study that the link between bone and implanting avoids osteolysis conjugated to the friction debris which has become more useful with HA coating. Therefore, in this study, as an alternative method to the self-locking intramedullar nails (IMN), in order to provide stability both ends (25 mm) of IMNs have been coated with micro and nano scale-HA, and were implanted in the left tibias of rabbits where the fractures were created. Fixation, biological osteointegration, bone unions were shown comparatively by biomechanical tests and post-operative histopathologic evaluations after a period of six weeks.

2. Materials and methods

This in vivo study was executed after having received the approval of the Firat University Ethical Committee for Animal Experiments on 20.01.2011, nr: 2011/0-14. New Zealand rabbits (n = 15) with an average age of 8.6 months (ranging between 7–12 months) and average weight of 3.398 kg (min 3.012–max 4.236 kg) were used. The rabbits were kept at 22 °C in a large cage and well fed during in vivo experiments. The animals were separated in three groups and each animal was preoperatively examined. Group I was the control group (K, n = 5), standard intramedullar nailing was materialized in Group II. (MHA, n = 5) micro HA coated nails were applied for the nailing process on the rabbits and (NHA, n = 5) nano HA coated intramedullar nails were applied for the nailing process on the rabbits in Group III. Antibiotic prophylaxis was applied after the operation and on the postoperative third day (1st generation ceflosporin). Each experimental object was checked separately in their cages after the operation, and PVC bandages were applied on each experimental object and the bandages were renewed postoperatively on the 15th day. All the subject rabbits were euthanized postoperative on the 45th day. The left tibias of the rabbits were post- euthanasia disarticulated from their knee and foot joints. The soft tissues on the tibias were scraped from the bones. The tibias which were torn off from each experimental subject were signed and biomechanical analysis coupling with histopathologic examinations was performed.

2.1. Anaesthesia and preoperative arrangement

5 mg/kg/im dose 2% Xylazine hydrochloride (Rompun, Bayer) was used for sedation, 45 mg/kg/im dose 10% (Ketamine Ketamidor, Richter Pharma) of anaesthesia was weighed with high accuracy and was intramuscularly injected to anaesthetize the rabbits. The operation area was arranged by shaving and disinfecting the lower joint and the upper joint of the tibia while embracing them. The skin antiseptics benzalconium chloride 10% (Zefiran®, Vilsan) and povidone iodine 10% (Biokadin®, Adeka) were used for disinfection. The area was covered with sterile overlays.

2.2. Surgical technique

The access to the medial incision was started with the proximal of the medial condyle of the tibia, and was directed to the cranial through the middle line of the tibia and later to the caudal in the surroundings of the medial malleolus. The subcutaneous was also incised in the same line. The incision was done carefully to avoid damage on V. Saphena & N. Saphenous and the curial fascia on the medial side of the diaphysis. M. Tibialis cranialis ve M. Fleksor digitalis were released from the bone by retracting the incision of the fascia, along the edges of the medialis. The crural fascia was incised along the cranial edges of M. Tibialis cranialis for displaying the lateral cortex. The incision started from the tuberosity tibia, and it was protracted till the muscular tendinous part to the dis-

tal. The cranial tibial and long digital extensor muscles were retracted to the quadrilateral, for displaying the diaphysis. The retractor was placed by paying attention to the cranial tibial arterial which goes through the tibia and fibula.

2.4. Intramedullar nailing process

A drill with a diameter of 2.7 mm was used at proximal tibia retrograde on the comprised fracture line with a hand drill, and a little incision of approximately 0.5 cm was carried out on the area where the drill came out from the proximal. The drill was ejected from the distal of the tibia. The nails were then sent to the proximal of the fracture line as retrograde and were ejected from the skin incision. The nail was placed with efficiency after the fracture had been reduced, and the intraoperative stability control was materialized. The subcutaneous tissues of the contusion area were stitched with 3/0 polypropylene wires (Sterilen, SSM, Istanbul/ Turkey), and the skin was stitched with 2/0 silk wires (Sterilen, SSM, Istanbul/Turkey). The left leg was bandaged with PVC. Peniciline G 1.000.000 IU (Kristapen potassium crystalized, Deva Ltd., Istanbul) was applied on the contusion area after the operation.

Postoperative controls of the phenomenon were carried out daily. The radiographic controls were done at intervals of 15 days. 20 mg/kg Cefazolin sodium (Cefamezin 500 mg, Eczacıbaşı, Istanbul) and 3mg/kg ketoprofen (profenid 100 mg, Eczacıbaşı, Istanbul) were applied on the animals for 3 days. The rabbits were sacrificed after 6 weeks and the operated left tibias were disarticulated from the distal and proximal joints.

2.5. Coatings of IM nails

Intramedullary nails were HA coated with the sol gel method by using HA powders with (4×100 mm) micron (~25 μm) and nano particles (~25 nm), with the aim to increase the osteointegration rate at both ends and by procuring stabilization in tibial object fractures, as given in Fig. 1a–c. Tensile tests were materialized on fixed fracture (transverse) bones with IM nails with points which were coated with hydroxyapatite (HA) of various partied sizes. The IMN points (25 mm) before the coating were subjected to the sand blasting process (60 μm silica beads) and were washed with detergents and kept for drying. Later, the IMN samples were left inactive for three hours in 25% nitric acid and were subjected to the cleaning process for 15 minutes in distilled water in an ultrasonic cleaning machine.

The composition of coating sol was: 40% HA, 30% P_2O_5, 20% $CaCO_3$ and 10% KH_2PO_4. These inorganic compounds were mixed in a magnetic stirrer for approximately 15 minutes, till a homogenous mixture was obtained. The solution was removed from the magnetic stirrer and was subjected to the homogenization process in an ultrasonic homogenizer (Cole-Parmer-750W). After the gel had been obtained, the ends of intramedullary nail points were dipped (at a distance of 25 mm) for 2 seconds into the gel, and they were coated in this way (Fig. 1a–b). Later the coated IM nails are left in air for drying (24 hours). After drying, the sintering process was executed in a horizontal sintering furnace (Nauberterm) in a vacuum atmosphere at 5 °C/min heating rate. The coated sample groups are given in Table 1 and Table 2, and the coated intermedullary nail samples, which were acquired after the sintering process are shown in Fig. 1a–c.

2.6. Biomechanical tests

After sacrificing, the treated fractures by IMN' located at mid-diaphysis were subjected to pull out tests by a universal tensile test machine (Shimadzu AG-X 50 kN) with a deformation speed of 2 mm/min. Before the tests, bone samples at both ends were placed in cylindrical polyester resin moulds from the epiphysis zones for sustaining anchorage of the tensile test machine jaws and mounted tibial ends were dried for approximately 12 hours. The test results of each group consisting of three samples are summarized in Table 2.

2.7. Radiology and histopathology

After radiologic X-ray and clinical examinations all the tibias were kept in the 10% formaldehyde solution for two weeks, and later decalcified in the Bouin's solution for two days (in 10% acetic acid, 80% NaCl and 10% formalin solution). For histopathologic examinations, the samples which were put into paraffin blocks were chopped in longitudinal parts of 3 microns. The samples were painted with hematoxylin eosin. The histopathologic classification of healing was made by Huo et al. in accordance with the histopathologic healing scale [28].

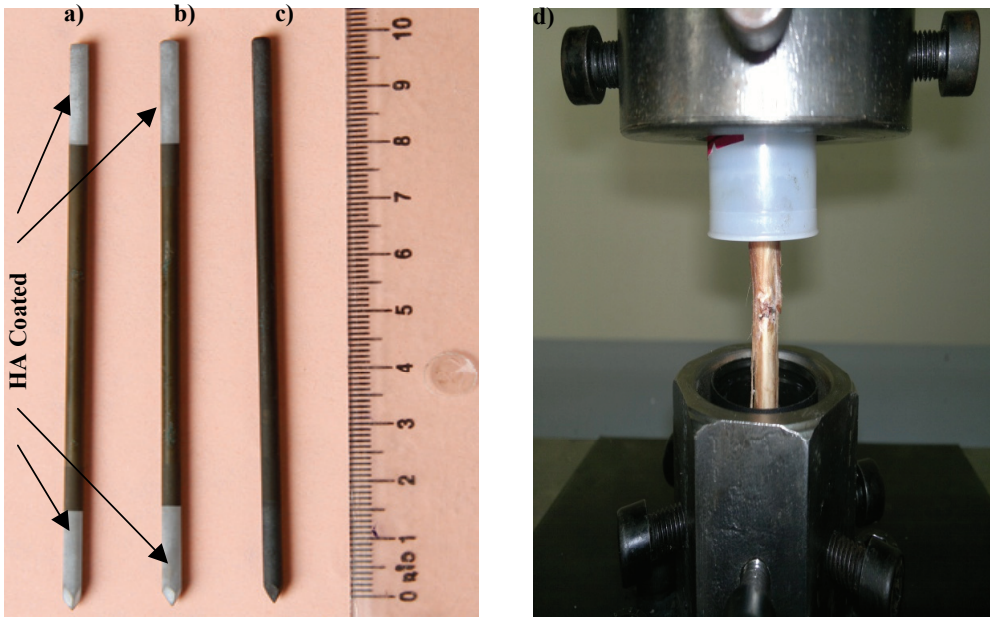


Fig. 1. Intramedular nails (IMN): (a) NHA coated IMN, (b) MHA coated IMN, (c) uncoated standard IMN (control group), (d) tensile test of IM nailed rabbit tibia

Table 1. Radiologic evaluation of fracture unions

Radiographic fracture evaluation		Control group Group I	MHA group Group II	NHA group Group III	*P* value
		n (%)	*n* (%)	*n* (%)	
Front-back radiography	No union	1(%100)	0	0	0.382*
	Possible union	4(%36.4)	4(%36.4)	3(%27.2)	
	Complete union	0	1(%33.3)	2(%66.7)	
Lateral radiography	No union	2(%66.7)	1(%3.33)	0	0.373*
	Possible union	3(%37.5)	2(%25)	3(%37.5)	
	Complete union	0	2(%50)	2(%50)	

* Ki-square test.

Table 2. Average comparisons of tensile test results between groups

	Control group ($n = 5$)	MHA group ($n = 5$)	NHA group ($n = 5$)	*P* value
	Average±s.inequality	Average±s.inequality	Average±s.inequality	
Tensile test	9.10±2.47	14.37±2.12	14.80±3.37	0.026**

** Kruskal–Wallis test.

2.8. Statistics

The SPSS 17 inc software was applied for statistical analyses. Supplementary statistical methods (Average, Standard inequality) were applied to evaluate the data. The Kruskal–Wallis test was applied for comparison between groups and was applied for comparing data between groups and the related groups were evaluated with the Wilcoxon test. The results were evaluated in the 95% trust portion, meaningfulness was at $p < 0.05$ level.

3. Results

The results of our prospective *in vivo* experimental pull out tests, radiologic and histopathologic investi-

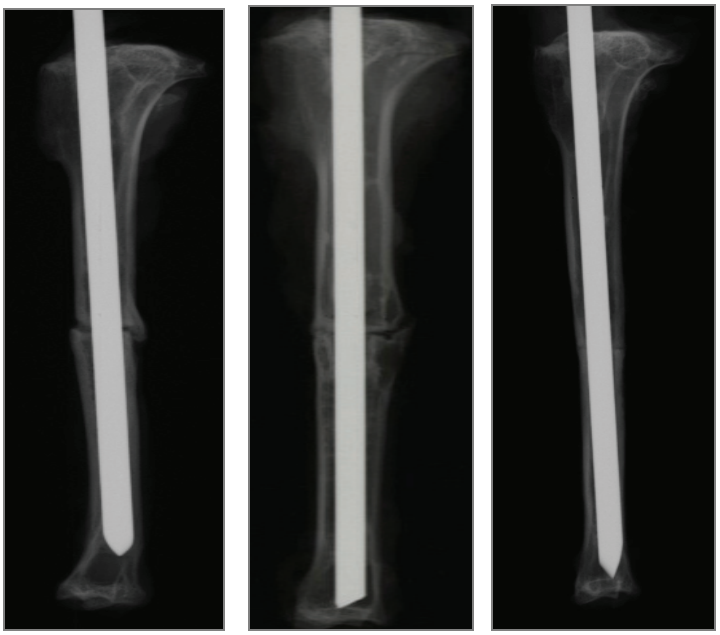
gations revealed the extended indications compared to standard tibial nails.

3.1. Clinical

The tibias were examined in a macroscopic manner after the radiologic examination. The scorings used were such as no action as 2, action in one single plan as 1, and action in two plans was evaluated as 0. There was not any trace of a statistical relation between the clinical examination symptoms and the groups ($p > 0.05$). All the rabbits that did not experience any union ($n = 2$) were in the control group. Medium grade union ($n = 11$) 27.2% of the rabbits ($n = 3$) were in the control group, ($n = 4$) 36.4% of them were in the MHA group, ($n = 4$) 36.4% in the NHA group. In total, 50% of the rabbits ($n = 1$) with complete union ($n = 2$) were observed in the MHA group and ($n = 1$) 50% of them were in the NHA group.

3.2. Radiology

As illustrated in Fig. 2a–c, the front-back and lateral plan backbone radiographies were taken after the left tibias. All the graphs were scored by a group of orthopaedists who independent of the experiment



a) b) c)

Fig. 2. Comparisonal X-ray views (taken after 45 days post operation) of fracture unions treated with IMN: (a) uncoated control IMN, (b) MHA coated IMN, (c) NHA coated IMN

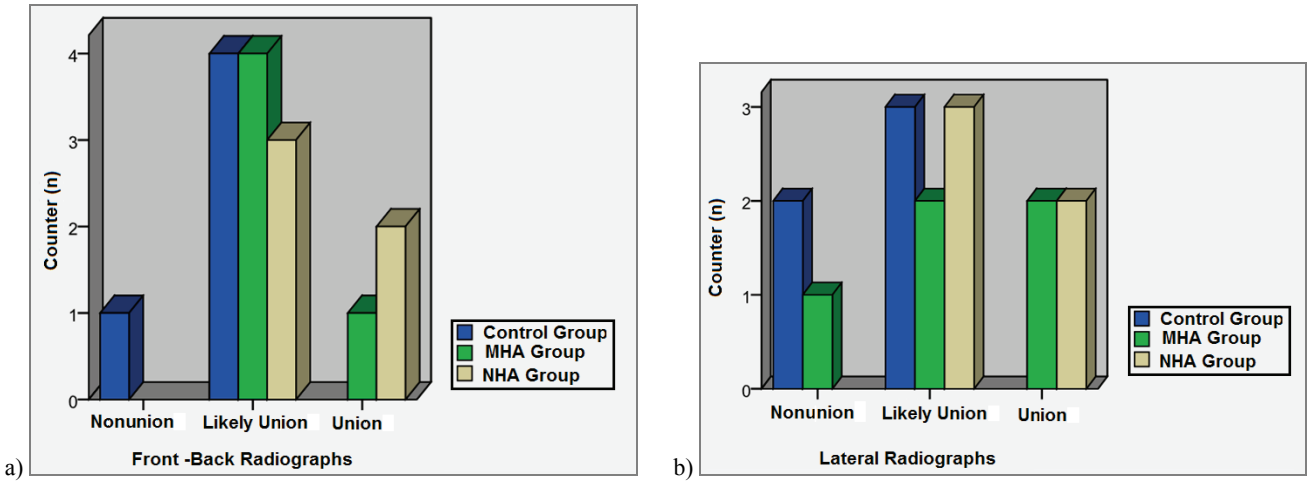


Fig. 3. X-ray radiographic fracture union assessments between the groups: (a) front-back radiographs, (b) lateral radiographs

classified in accordance with the Goldberg classification [29]. Radiographic comparative X-ray views of fracture unions of coated and uncoated tibial samples are illustrated in Fig. 3a–c.

3.3. Biomechanics

In order to demonstrate the new alternative fixation method the nailed tibias which were treated by HA-coatings were subjected to pull out tests (Fig. 1d). The results of such tests will be the indication of fixation level. Three groups of specimen (Table 2) have been tested and the stress-elongation (%) curves are presented in Fig. 4 in comparison with Control, MHA and NHA. The graphical comparative plots of all the groups are shown in Fig. 5.

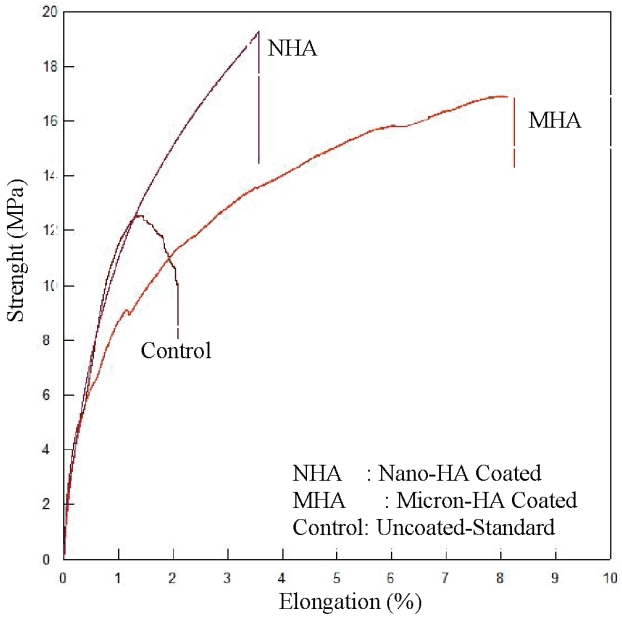


Fig. 4. Maximum strength-elongation (%) curves of the groups

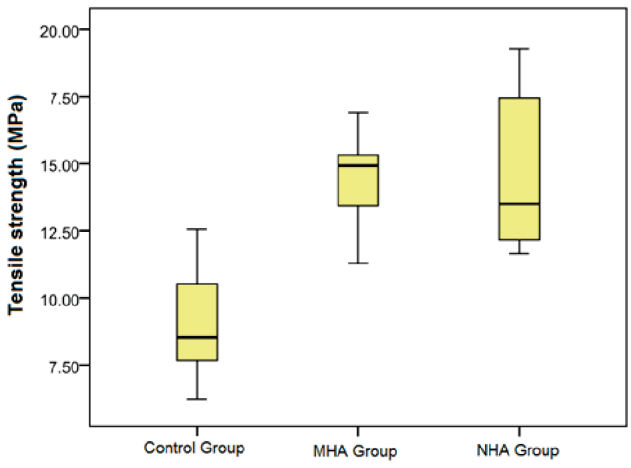


Fig. 5. Variation of tensile strengths of the IM nailing groups

3.4. Histopathology

In order to observe healing fractures, newly generated bones and the changes in bone tissues near to the IMN ends and at fracture zones, histopathology examinations were performed in addition to biomechanical tests. Histopathology analyses were divided into two groups to display the fracture area and the area near to the IMN ends where the changes in the bone tissues occurred.

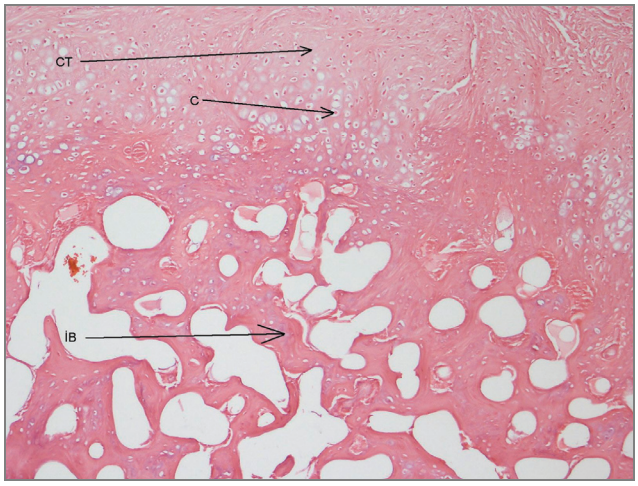


Fig. 6. Histogram of the 3rd level of callus tissue of the control group (uncoated ends) of IMN at fracture zone: CT (cartilage tissue), C (condrosite), IB (immature bone)

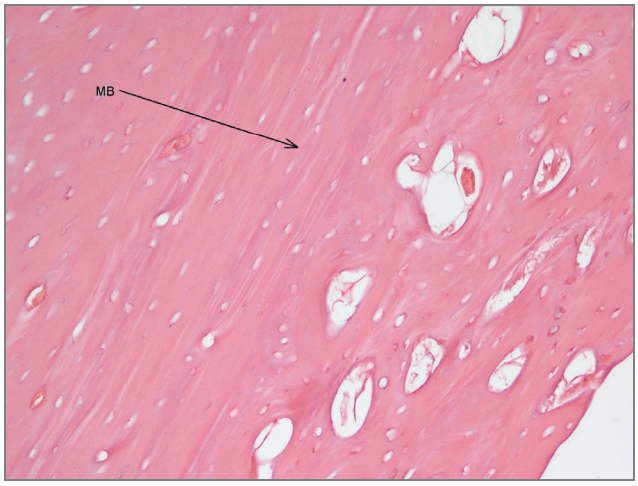


Fig. 7. Histogram of the 3rd level of callus tissue (X200) of NHA group at fracture zone: MB (mature bone)

The histograms which were obtained as a result of examining histopathologic incision samples of three groups (uncoated, micro scale-HA coated IMN and nano scale-HA coated IMN) are shown in Figs. 6–8. The histograms which are obtained from the histopathologic examination of the bone incision samples

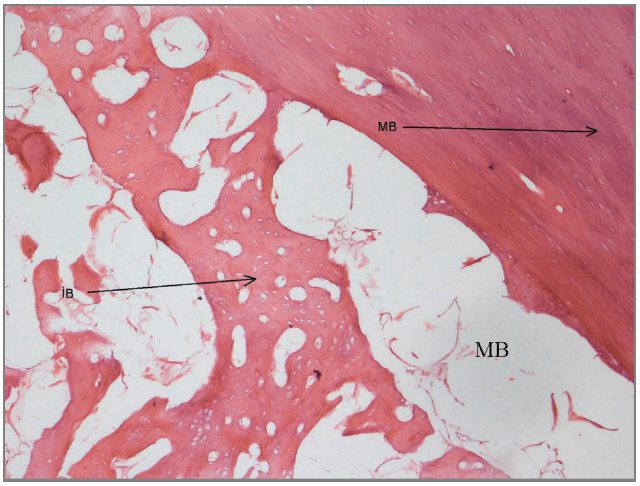


Fig. 8. Histogram of the 3rd level of callus tissue (X200) of MHA group at fracture zone: MB (mature bone), IB (immature bone)

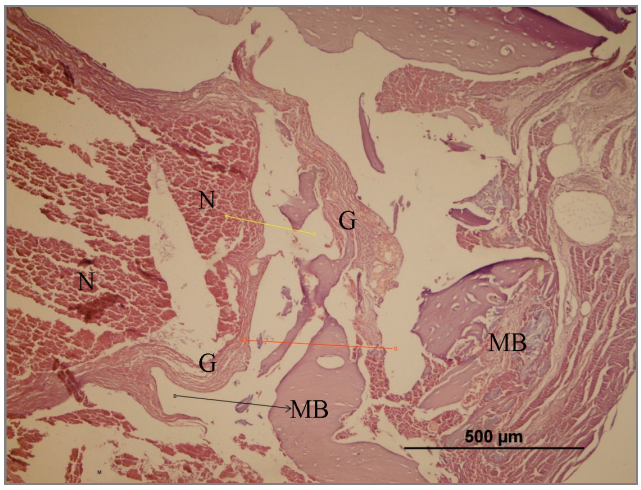


Fig. 10. Histogram of the 3rd level of callus tissue of the MHA group (uncoated ends) of IMN at the zone near to coated IMN ends: MB (mature bone), N (necrotic tissue), G (granulation), N (necrotic tissue)

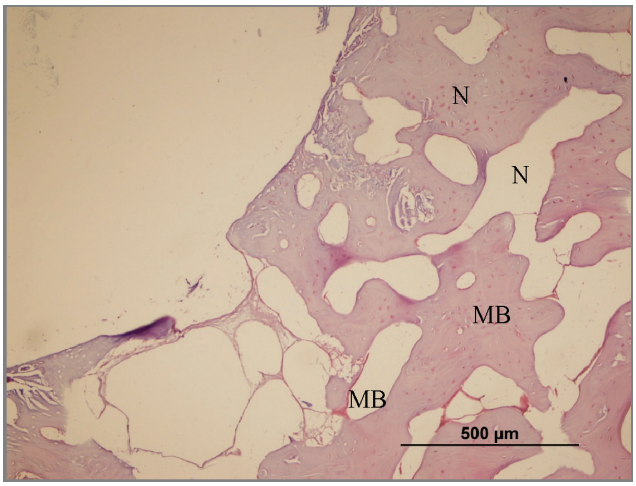


Fig. 9. Histogram of the 3rd level of callus tissue of the control group (uncoated ends) of IMN at the zone near to coated IMN ends: MB (mature bone), N (necrotic tissue)

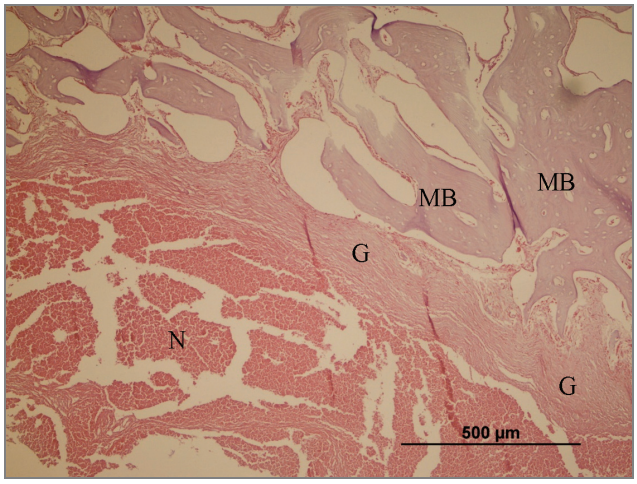


Fig. 11. Histogram of the 3rd level of callus tissue of the NHA group (uncoated ends) of IMN at the zone near to coated IMN ends: MB (mature bone), N (necrotic tissue), G (granulation), N (necrotic tissue)

taken from the areas near to the HA coated IMN ends are also shown in Figs. 9–11. The histogram in Fig. 6 displays the bone tissue of the control group (uncoated) which was generated near to the fracture zone. The histograms in Figs. 7 and 8 of the micro and nano scale-HA coated IMNs are displayed, respectively. The histograms which demonstrate the bone generation in the areas near the IMN ends, are displayed as control (uncoated), micro-HA coated and nano-HA coated in Figs. 9, 10 and 11, respectively. The abbreviations displayed with arrows on tissues are as follows: the Fibrous tissue (FT), Condroit tissue (CT), and Woven tissue (WT), MB (mature bone), IB (immature bone), CT (Cartilage tissue) and C (condrosite).

4. Discussion

4.1. Radiology

As the radiologic evaluation has shown in Fig. 2a, b, there was no tibiae encountered without union in the front-back radiography (n = 1) which belonged to the control group. Union was observed (n = 11) in 36.4% of the rabbits (n = 4) for the control group, (n = 4) 36.4% for the MHA group, and (n = 3) 27.2% for the NHA group, respectively. A complete union was detected (n = 3) in 33.3% of the tibias (n = 1) which belonged to the MHA group. No union in the

front-back radiography was observed in (n = 3) 66.7% of the rabbits, (n = 2) in the control group and (n = 1) 33.3% in the MHA group. The union was detected in (n = 8) 37.5% of the rabbits, (n = 3) 37.5% in the control group, (n = 2) 25% in the MHA group and (n = 3) 37.5% in the NHA group. Complete union was observed (n = 4) in 50% of the rabbits (n = 2), in which 50% of them were in the MHA group and (n = 2) 50% were in the NHA group. However, no statistical relation was encountered between the front-back radiography symptoms and the groups ($p > 0.05$).

4.2. Biomechanics

It was observed in earlier research that even a micro action as little as 100 μm between the bone and the implant can cause the generation of a fibrosis membrane by preventing bone development. Soballe et al. [29] showed that the fibrosis tissue in the surrounding of the implant was ossified pursuant to the burden imposed to the HA-coating. Both groups with fractured bones which were fixated with HA coated intramedullar nails used in Micron (30 μm) and Nano (25 nm) particle sizes, have also shown higher resistance of tensile stress in accordance with the fractured bones which were fixated with the uncoated nails (control group). Also as shown in Fig. 4, the increase in tensile stress is 58% for the MHA group and 63% for the NHA group compared to the control group. These are very positive results in terms of biomechanics, fixation, and the union grade.

The connection between HA coating and the ligament will, at the same time, restrict the movements of the nail within the bone, during the healing process and because it will increase the fracture stability in the diaphysis, it will naturally accelerate the healing process of the fracture. Hence, the connection between the nails and the bone will be stronger in fractured bones where intramedullar nails are fixated, at fracture area diaphysis, and in the areas near to epiphysis.

The control group samples contain the fractured bones which are fixated with uncoated standard intramedullar nails. The result obtained from the NHA group samples resembled the results that were obtained from the other groups, and the highest biomechanical resistance was reached in this group. However, this increase is 63% in accordance with the control group samples. The increase in tensile resistance was about 63% compared to uncoated ones.

The average differences (Fig. 5) were found statistically significant ($p < 0.05$) between the groups of uncoated, coated by MHA and NHA. The average tensile stress of the control group was 9.10 ± 2.47 MPa, as it was 14.37 ± 2.12 for MHA group and 14.8 ± 3.37 MPa for NHA group. The distinction between the groups was also found to be statistically significant. Statistical distinction was observed between the MHA group and the other two groups ($p < 0.05$). The average strength of the MHA group (6.76 MPa ± 1.60) was found to be higher than the averages of the control group (3.65 ± 1.56), and the NHA group (2.74 ± 1.53). This result may be interpreted in this way: positive contribution was supplied to the healing process and consequently with a better fixation during the healing period at the fracture zone in the diaphysis remained more stable by using the HA coated ends (25 mm) of IMNs.

4.3. Histopathology

A sample of histopathological incision of the control group (without coating) taken from the fracture zone (Fig. 1d) is shown in Fig. 6. A healing fracture consisting of an immature bone and cartilage – shown with arrows was observed. A coloured incision painted with hemotoxylin-eosin zoomed in the 3rd phase callus tissue was histopathologically observed. Chondroid and fibrous tissue generation on equal levels was observed in the histogram. The dominant image in the histopathological incisions obtained from the experimental subjects in the control group consists of immature bone (IM) and cartilage tissues (CT), as seen in the histogram. As is well known, fracture healing on insufficient stability conditions occurs mostly by immature bones and cartilages. Thus, the uncoated IM nails we used in the control group did not give sufficient stability and we can conclude from this that it has an unfavourable impact on the healing process of a fracture.

A histopathologic incision (hemotoxylin-eosin) which belongs to the nano-HA coated (NHA) group taken from the fracture zone is shown in Fig. 7. The mature lamellas which are indicated on the histogram reveal that there is a bone generation and a healing fracture in the 3rd phase callus tissue. Fracture healing consisting of mature (MB) and, from place to place, immature bones (IB) is observed in most of the histopathologic incisions which belong to the NHA group. This situation, in fact, validates and supports the positive results obtained from the biomechanical tests. Finally, we can emphasize from these results that the HA coated IM-nails provided higher stability which is crucial for fracture healing. The fracture healing of experimental subjects in the control group seems

– even if just a pinch – to be less developed when compared with other coated IMs, in accordance with the radiography evaluations. Better union symptoms (more callus) were observed in the radiographies of the MHA group as demonstrated in Fig. 8. An incision coloured with hematoxyl-eosin was observed histopathologically in histogram with magnification X40 in the 7th phase callus maturation for the micro-HA coated IMN group (MHA). As is also shown in the histogram, there is a woven bone which has not yet completed its maturation where the chondroid tissue appears in small quantities. The dominant union type is a union type which consists of mature and, from place to place immature bones, as it was already shown in the histopathologic incision of the MHA group. The ligaments observed in this histogram suggest that better union was obtained in comparison with the control group. With the stability resembling the tensile test results, a better fracture union process related with this was obtained. An incision coloured with hematoxyl-eosin was observed when it is focused in the 10th phase callus maturation. As seen in Fig. 10, the cortical and trabecular ligament generation can be observed.

Tissues derived from the sacrificed rabbits after six weeks in areas near to both IMN ends, in Group II are shown in Figs. 9–11. The medullar cavity has been narrowed mechanically and has contributed to the stability of the implant and the fractured line. From the biomechanical test results, the fixation and hence, the fracture bone union have been found significantly meaningful for the coated materials. In the control histogram shown in Fig. 9, MC (medullar cavity), N (necrosis), MB (mature bone) were observed. Necrotic tissues were observed in the surrounding of the mature bone neighbouring the medullar cavity and new bone generation has also been observed in control group. The ligament was obtained from the micro-scale HA coated ends of the IMN ends (shown in Fig. 1a–c by arrows), relevant tissues like B (bone), N (necrosis), G (granulation tissue) M (medulla) were observed. Figure 10 shows the histogram of MHA coated IM nail end. The new bone generation, from place to place and granulation tissue, necrotic tissues are also observed in the surrounding of the implant, and new bone generation in neighbouring to IMN end is also seen. Figure 11 shows the histogram of nano scale-HA coated IM nails near to the coated ends (25 mm). From the nano scale-HA coated IM nail end shows a certain granulation tissue generation in the implant material surrounding the histogram and little necrotic areas are observed.

Although limited new bone generation in the IM nails surrounding of the control group samples shown in the histogram (Fig. 9), granulation tissues in the NHA group, from place to place, new bone generation and granulation tissues in the MHA group were observed. These good results can be explained by the osteoinductive impacts of HA coatings and the HA coated surfaces have triggered new bone generation.

5. Conclusions

As a result of biomechanical tests and histopathological analyses, it has been observed that for the ends (25 mm) of the intramedullar nails coated with HA which is in contact with the medullar bone, the fixation was found to be statistically significant. It has been observed that the HA particle size (micron or nano) does not show a meaningful distinction biomechanically. However, NHA group IM nails provided slightly higher pull-out strength or fixation than those from MHA group, but certainly much higher than uncoated control group. From the histopathological examination, the dominant image obtained in the control group (uncoated) consists of immature bone and cartilage tissues. Fracture healing consists of mature and, from place to place, immature bones in most of the histopathologic incisions for the NHA group. Compared to control group, much better union symptoms (more callus) were observed in the radiographies of the MHA and NHA group. However, further studies in comparison with locking intramedullar nailing may be helpful to show advantages of those methods over each other. Furthermore, *in vivo* experiments have to be conducted in longer periods for better evaluation of fracture healing.

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