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Survival of percutaneous implants under various mechanical loading to the bone

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Objective The purpose of the study was to explore bone formation processes and survival of percutaneous implants under various external compression of the bone. **Material and methods** 30 chinchilla male rabbits were used in the study. Tibia of the species was amputated at the upper third and an implant was surgically implanted with the distal part extending through the skin. A compression device was attached to the implant and loading provided to the bone next day after surgery. Five magnitudes of compression loading were used for animals subdivided into 5 groups comprising 6 species in each of the groups. Compression device with constantly maintained loading was attached to the limb during 6 weeks. **Results** Animals of groups I and II showed no case of the implant falling out throughout the whole period of observation. An implant fell out of the bone in one species of Group III after 56-day implantation, two and four species of Groups IV and V, correspondingly, 3 to 4 days after removal of compression device. Osseointegration was shown to improve in species of Groups I and II due to active angiogenesis in peri-implantation area. **Conclusion** Therefore loads of greater than 105260 H/m^2 applied to the bone result in decreased implant osseointegration whereas less intensive loading tends to improve osseointegration.
Keywords: implant, survival, osseointegration

Extensive research has been conducted in regenerative medicine to substantiate application technologies for a variety of metal implants to be used in orthopaedics and prosthetics [1, 2]. Implant stability/survival is one of major concerns since failures remain a severe complication resulting from pathologies that require implant ingrowth into the bone (total joint replacement, bone defect repair, fractures, etc.) [3, 4].

Metal implant instability appears to be a serious problem for the patients who need osteointegration technologies for prosthesis after limb amputation [5–7]. Percutaneous osseointegrated prosthesis is reported to be dependent on several factors including the geometry, plasticity, topology; technique used to mould the surface pattern; mechanical force applied to the bone through the implant [8–10].

In our opinion, the load on implant can be considered the main cause of unstable percutaneous prostheses and can either result in osteolysis at the bone-implant interface or stimulation of osseointegration processes at the surface depending on the intensity of the force.

Objective The purpose of the study was to explore bone formation processes and survival of percutaneous implants under various external compression loads on the bone.

MATERIAL AND METHODS

Thirty chinchilla male rabbits with an average weight of 3.4 ± 0.2 kg were used in the study. The age ranged from 6 to 10 months. The animals were obtained from breeding and supply facilities OAO Synthes (Kurgan). Microbiological status of the animals was defined as conventional.

Tibia of the species was amputated at the upper third by one operating surgeon and an implant (utility model patent № 152558) was surgically implanted with the distal part extending through the skin (**Fig. 1, a**). Then an author's compression device was attached to the implant (**Fig. 1, b**).

Loading to the bone was provided with the compression device next day after surgery. Five magnitudes of compression loading measured 52630 N/m^2, 105260 N/m^2, 157890 N/m^2, 210520 N/m^2, 263150 N/m^2. So, the animals were subdivided into 5 experimental groups comprising 6 species in each of the groups. Compression device with constantly maintained loading was attached to the limb during 6 weeks.

A clinical fluctuation test was performed daily for the distal part of the implant to monitor implant stability, assess gait pattern, general mobility and postimplantation period length prior to signs of implant loosening. Radiographs were taken once every two weeks.

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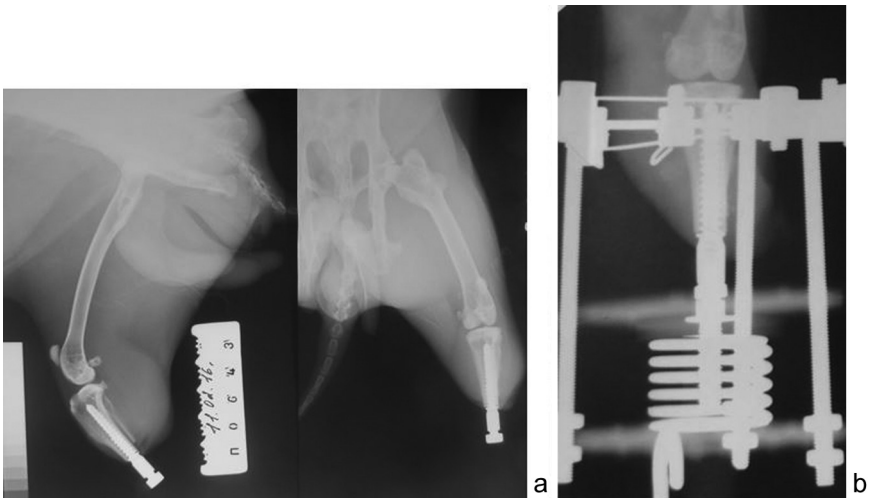

Fig. 1 Postoperative radiographs of pelvic limb showing (**a**) the implant placed in tibia, (**b**) compression device attached

Implant stability measurements were produced with wireless hand-held Periotest-M device (Germany) to assess damping characteristics the next day of implantation. The damping capacity was measured once every two days.

Animals of any group were sacrificed once they showed signs of implant loosening with clinical-radiological and biomechanical assessments. Maximal observation period was 26 weeks according to GOST ISO 10993-6-2011 "Medical devices. Biological evaluation of medical devices. Part 6. Test for local effects after implantation". Animals were sacrificed at the end of 26-week implantation even with no signs of instability.

Implant survival was defined as the period (days) between implantation and appearance of signs of implant instability. Bone-implant block was fixed in 10 % neutral buffered formalin after euthanasia and cut longitudinally so that one part contained integrated implant. The saw cut and the implant were dehydrated and embedded in camphene. The specimen was dried in the air after distillation and assessed with scanning microscope and electron probe micro-analyzer. Calcium and phosphorus location and concentrations in the tissue substrate adhered to the implant surface were determined with electron probe X-ray micro-analyzer "INKA Energy 200" (Oxford Instruments Analytical). Jeol JSM-840 scanning electronic microscope (Japan) was used to examine structure of the specimen.

Another half of the bone-implant block was decalcified and embedded in paraffin. Reichert sledge microtome (Germany) was used to cut sections for histological examination and the specimen were stained with hematoxylin and eosin and Masson trichrome and osteopontin polyclonal antibodies were employed for histochemical staining (protocol and reagents from Abcam, Germany). Histostructure of the tissues in the bone-implant block was examined with light microscopy using AxioScope A1 stereomicroscope, AxioCam ICc 5 digital camera and Carl Zeiss MicroImaging GmbH's ZEN software (blue edition, Germany).

The animals were housed in a kennel facility of the Russian Ilizarov Scientific Center "Restorative Traumatology and Orthopaedics" (RISC "RTO") Ministry of Health of the Russian Federation. Rabbits were kept singly in cages without shelves. The cages were equipped with food and water tanks. Non-conifer wood scraps were used for flooring. Wet cage cleaning was provided every day. The diets were given once daily and pure sterile drinking water supplied in unlimited amounts. A 21-day quarantine was imposed on animals prior to the experimental use. The use of the animals in research was governed by SP 2.2.1.3218-14 "Public health requirements for design, facilities and maintenance of experimental and biological clinics/kennels" in compliance with GOST (National State Standard) 33215-2014 "Guide for the care and use of laboratory animals. Regulations of housing, management and organization of procedures", GOST 33216-2014 "Guide for the care and use of laboratory animals. Regulations of the care and use of laboratory rodents and rabbits".

The research project received ethics approval of the Research Ethics Board of the RISC "RTO" Ministry of Health of the Russian Federation.

RESULTS

Clinical findings. Clinical condition of the rabbits was found to be satisfactory after implantation in both groups. The supporting function of the limb normally restored after 4 to 5 postoperative days and persisted in all the animals throughout the observation period. Four rabbits (one in each of the Groups 1, 3, 4, 5) developed pin tract infection that was treated with anticeptics. No cases of implant fallout were observed in Groups 1 and 2 throughout the whole period of observation (**Fig. 2, a**). The implant fell out of the bone in one animal of Group 3 at 56 days of implantation (**Fig. 2, b**). The implant fell out of the bone in two animals of Group 4 and four animals of Group 5 at 3 to 4 days following compression device removal (45–46 days after implantation) with identical radiological findings.

Cumulative data of implant survival in animals that accomplished the research project are presented in Table 1.

Biomechanical evaluation. Measurements of implant stability are presented in Table 2. Greater instability was observed in animals of Group 5 at 6 weeks of implantation. Implant damping capacity was at zero values in rabbits of Groups 1–3 at 12 weeks of implantation that indicated to a high level of osseointegration. Group 4 showed slightly less measurements of damping characteristics with a medium level of integration at bone-implant interface.

Histological assessment. Mesobrochate structured trabecular bone connecting the inner surface of compact layer and the surface of integrated implant was noted to form in animals with stable implant at 26 weeks of osseointegration (**Fig. 3, a, c, e**).

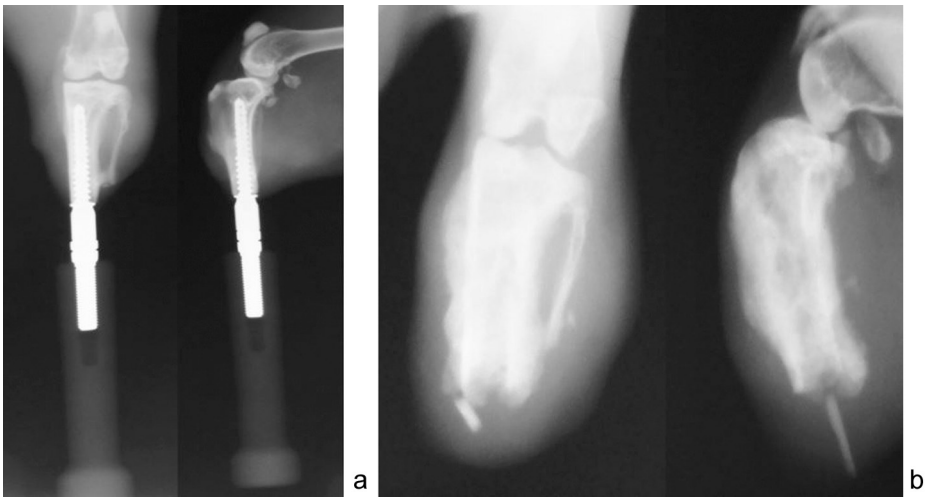

Fig. 2 Radiographs of rabbits' tibiae: (**a**) at 84 postoperative days (Group 1); (**b**) after implant fallout

Table 1

Implant survival (number of prosthesis/number of animals; survival rate) in rabbits that accomplished the research project

Group	Weeks after implantation				
	3	6	12	20	26
Group 1	6/6; 1.0	6/6; 1.0	6/6; 1.0	6/6; 1.0	6/6; 1.0
Group 2	6/6; 1.0	6/6; 1.0	6/6; 1.0	6/6; 1.0	6/6; 1.0
Group 3	6/6; 1.0	6/6; 1.0	5/6; 0.83	5/6; 0.83	5/6; 0.83
Group 4	6/6; 1.0	6/6; 1.0	4/6; 0.67	4/6; 0.67	4/6; 0.67
Group 5	6/6; 1.0	6/6; 1.0	2/6; 0.33	2/6; 0.33	2/6; 0.33

Table 2

Dynamics in implant damping capacity (conventional units) in rabbits that accomplished the research project (mean arithmetic ± standard deviation)

Group	Weeks after implantation				
	3	6	12	20	26
Group 1	221 ± 2.3	12.1 ± 2.1	- 2.8 ± 0.4	- 3.8 ± 1.2	- 6.2 ± 2.1
Group 2	24.4 ± 4.1	15.3 ± 2.7	- 1.6 ± 0.3	- 3.2 ± 0.6	- 5.4 ± 1.2
Group 3	28.2 ± 1.1	22.3 ± 1.4	1.6 ± 0.7	0.3 ± 0.3	- 1.4 ± 1.5
Group 4	27.4 ± 2.2	23.5 ± 1.3	3.3 ± 0.3	2.2 ± 0.4	1.1 ± 0.5
Group 5	35.6 ± 4.2	29.2 ± 1.4	5.3 ± 1.2	0.5 ± 0.4	-1.1 ± 0.7

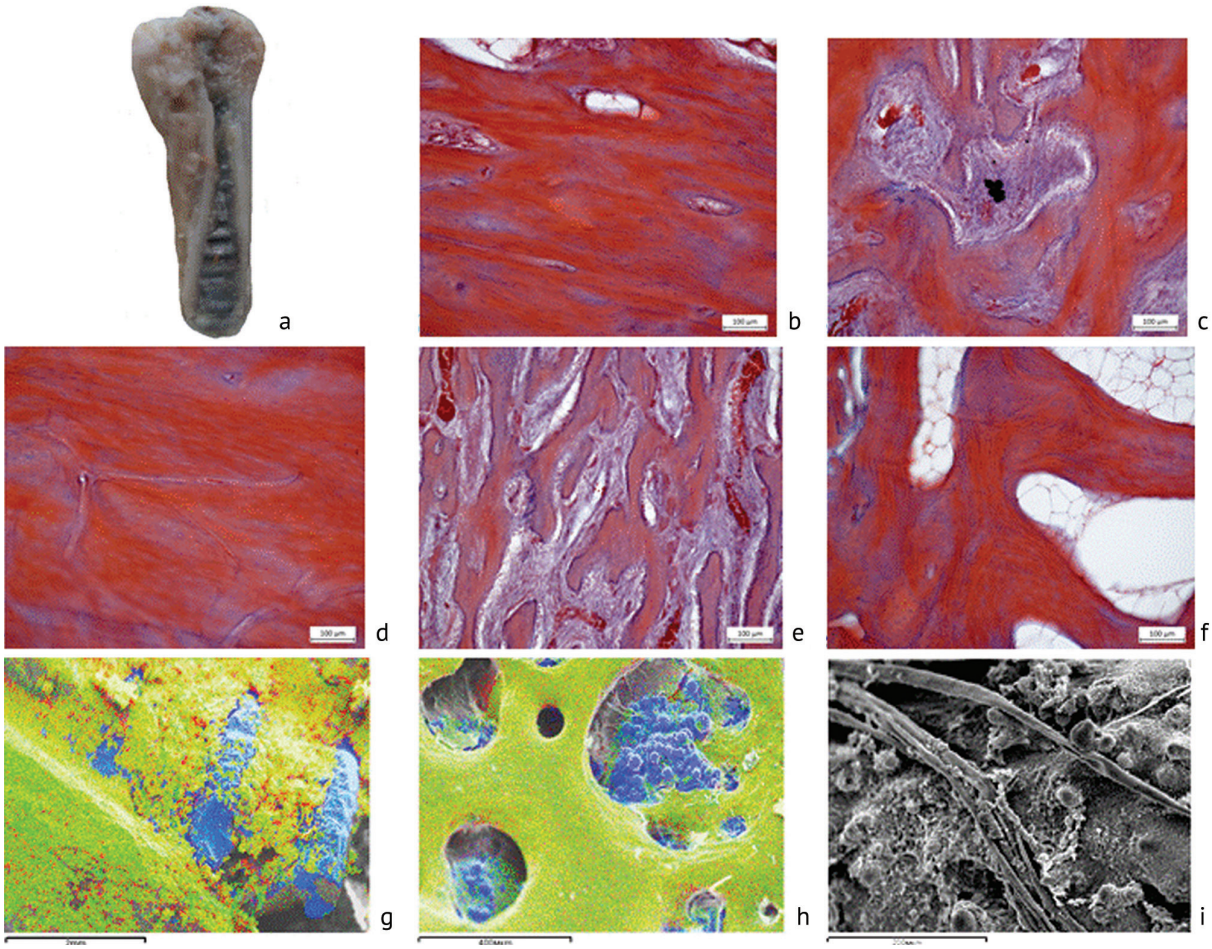

Fig. 3 Bone-implant block formed at 26 weeks of the experiment. Group 1: (**a**) tibial saw cut of a rabbit with integrated implant; (**b**) compact plate with widened Haversian lumens in the distal portion of the stump. Masson's trichrome stain; magnification 200×; (**c**) microphoto of tissue components forming between compact plate and the implant in the distal part of the stump. Masson's trichrome stain; magnification 200×; (**d**) compact plate and widened Haversian lumens in mid stump. Masson's trichrome stain; magnification 200×; (**e**) microphoto of tissue components forming between compact bone and the implant in mid stump. Masson's trichrome stain; magnification 200×; (**f**) microphoto of tissue components forming between compact bone and the implant in the proximal part of the stump. Masson's trichrome stain; magnification 200×; (**g**) distribution map of Ca (red) and P (green) in the tissue substrate adhered to the surface of mid implant (Ti, blue), magnification 800×; (**h**) distribution map of Ca (red) and P (green) in the tissue substrate adhered to the surface of the proximal part of the implant (Ti, blue), magnification 140×; (**i**) bone matrix and microvessels on the surface of mid implant. SEM, magnification 300×

Spongy fibrous connective tissue, isles of hemopoiesis (**Fig. 3, c**) and red bone marrow were observed in intertrabecular spaces of the distal and middle parts of bone-implant block. Red-yellow and yellow bone marrow was seen proximally (**Fig. 3, f**). Compact plate was typically structured (**Fig. 3, d**). No signs of inflammation around the implant could be noted in majority of animals. Homogeneous tissue substrate was detected on threading ribs and cavities of the implant with Ca and P identified with electron probe X-ray micro-analyzer in all the animals (**Fig. 3, f, g**).

Signs of osteogenesis were more evident in the distal part of bone-implant block in Group 1 with greater expression of osteoponin in immune histochemical reaction with osteoponin polyclonal antibodies (**Fig. 4**).

Apart from osteogenesis in the group with stable implants periosteal and endosteal compactization was also more expressed in the animals with osteoclastic resorption being combined with intense osteo- and angiogenesis (**Fig. 5**). These processes were thought to be caused by compression forces that also facilitated implant particle exfoliation and migration into the tissues (**Fig. 5, c**).

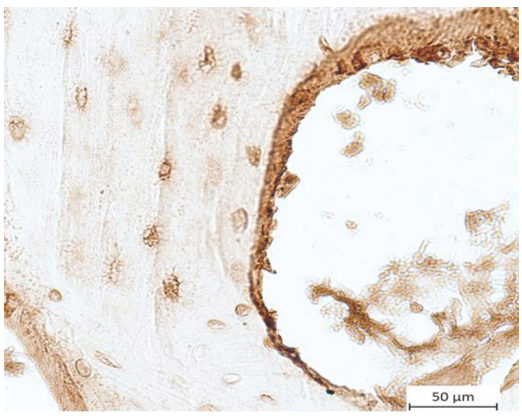

Fig. 4 Expression of osteopontin in compact plate in the distal part of the stump at 26 weeks of the experiment. Group 1. Immune histochemical reaction with osteopontin polyclonal antibodies. Magnification 400×

Osteotropic elements (**Fig. 6**) were observed on the surface of vessels adhered to implants and in perivascular cells that indicated to differentiation of multipotent perivascular cells in osteogenic manner in this environment.

Compact plate appeared to be osteoporotic in cases of implant fallout (**Fig. 7**). Implantation constructs were poorly held inside the bone. The implant surface contained scattered areas of tissue components with signs of adhesion and poor signs of mineralization that were seen in distribution maps of Ca and P in tissue substrate. Fat bone marrow, elements of hemopoiesis and foci of inflammatory infiltrate and fibrosis were observed in the gap between the implant and compact plate.

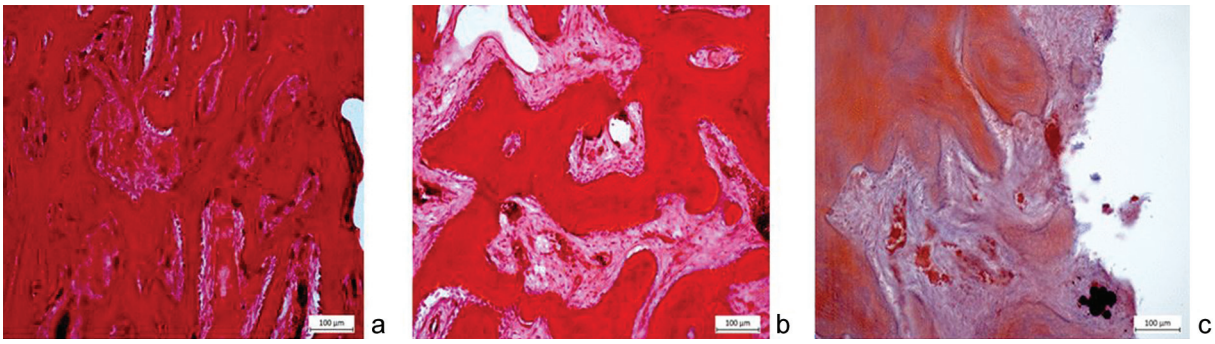

Fig. 5 Bone tissue at different parts of bone-implant block at 26 weeks of the experiment. Group 1: (**a**) periosteally formed bone tissue in the distal part of bone-implant block; (**b**) bone tissue formed between compact plate and the implant in mid stump; (**c**) an area between compact plate and the implant in the distal part of the stump. Particles of titanium powder can be viewed. Stain: Van Gieson's (a, b), Masson's (c). Magnification 200× (a, b, c)

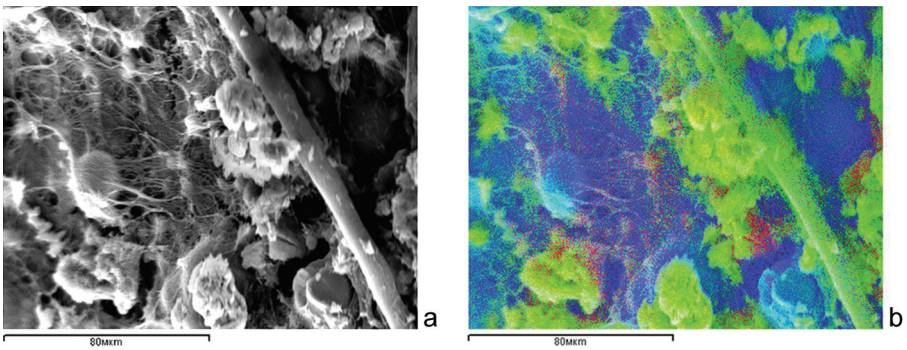

Fig. 6 Microvessel of a capillary type in tissue matrix on the surface of the middle part of the implant at 26 weeks of the experiment. Group 1: (**a**) electronic image. SEM; (**b**) distribution map of Ca (red) and P (green) in tissue substrate adhered to the surface of the middle part of the implant (Ti – blue). Magnification 650×

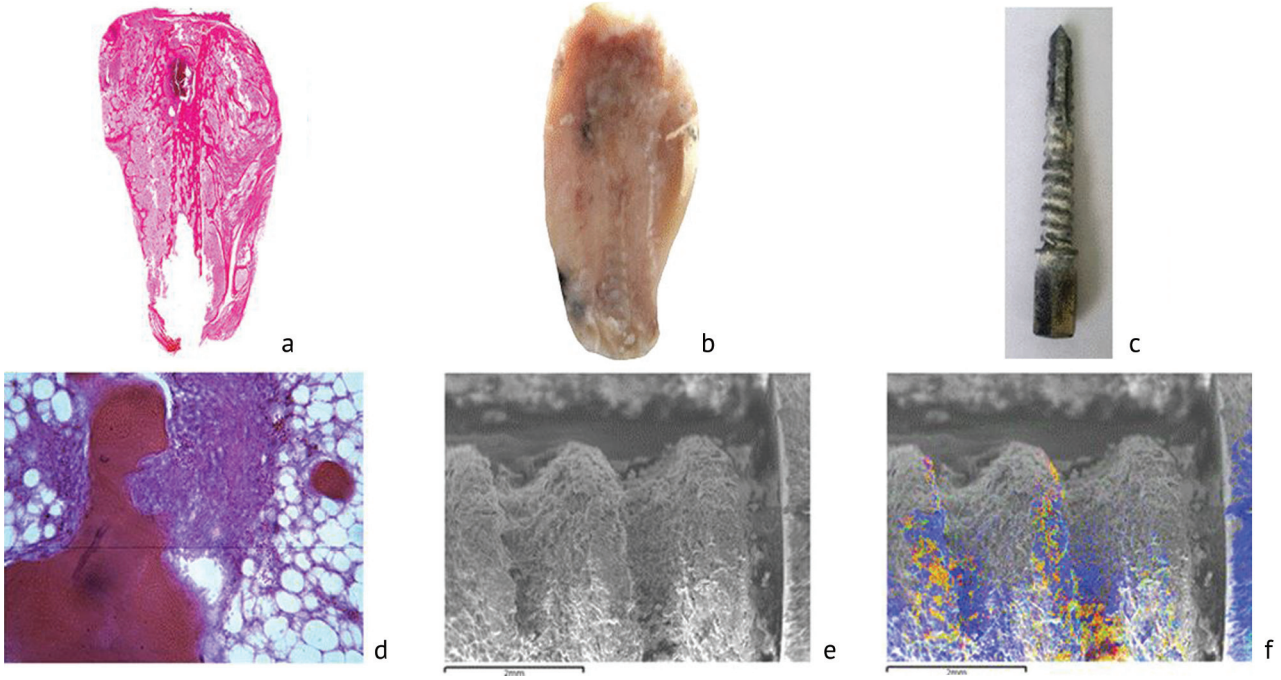

Fig. 7 Structural changes in tissue components of bony bed and the surface of implanted construct at 45 days of implantation. Group 5: (a) histotopogram of the saw cut of the rabbit's tibial stump. H&E stain. Magnification 1.5×; (b) saw cut of the tibial stump. Macropreparation. Magnification 200×; (c) microphoto of tissue components formed in the gap between compact plate and the implant. H&E stain. Magnification 200×; (d) macropreparations of the implant removed and saw cut of the bony bed; (e) electronic image of the distal part of the bone-implant block. SEM. Magnification 20×; (f) distribution map of Ca (red) and P (green) in the bone-implant block. Ti - blue. Magnification 20×

DISCUSSION

To summarize the findings we calculated the mean implant survival rate in days (the mean is not quite appropriate in the case but illustrative enough) that measured 182 days in Group 1 and 2, 161 in Group 3, 137 in Group 4 and 91 days in Group 5. Our results showed that different magnitudes of compression forces applied to the bone had different impact on osseointegration of percutaneous implants. We detected approximate values of loads that would positively (or at least not negatively) impact on osseointegration of the implants and ranged from 52630 to 105260 N/m^2. Compression forces of more than 105260 N/m^2 was shown to result in implant fallout within 26 weeks. Osseointegration failed in the majority of the cases with ultimate compression loading of 263150 M/m^2 and the implant fell out of the bone immediately after removal of compression device.

Histological assessment demonstrated that compression forces of 52630 N/m^2, 105260 N/m^2 applied in Groups 1 and 2 appeared to prevent porous changes in compact bone and contribute to osseointegration of the implanted titanium construct due to more intense angiogenesis in peri-implantation area with greater inflow of less differentiated cells being differentiated in osteogenic manner under mechanical forces.

This series suggests that a certain amount of compression applied to percutaneous implant can be considered as one of pre-requisites for the successful osseointegration. Quantitative minimum-maximum measurements of compression loading cannot be viewed as accurate due a small sample population and a small range of forces applied. However, the experimental model has no analogs reported in the literature and the findings can serve the starting characteristics of compression for further research in both experimental and clinical aspects. The unique character of the model allows no correlations in implant survival rate obtained in our series with that observed in clinical findings [9].

CONCLUSION

The findings of the research suggest that there is a correlation between the amount of compression forces applied to the bone through the implant and the quality of osseointegration. Loads of greater than 105260 H/m^2 applied to the bone were shown to result in decreased implant osseointegration whereas less intensive loading tended to improve osseointegration.

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