

**EFFECTS OF LOCAL APPLICATION OF PLATELET-RICH PLASMA AND GUIDED TISSUE REGENERATION ON STABILITY OF IMPLANTS**

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*Osseointegration is a result of cellular migration, differentiation, bone formation, and bone remodelling on the surface of an implant. Each of these processes depends on platelets and blood coagulum. Platelet-rich plasma (PRP) is used to improve osseointegration and stability of implants. It is a blood fraction produced in a centrifuge which contains a high concentration of platelets. Platelets contain numerous growth factors which influence tissue reactions.*

*The aim of the research is to test clinically the influence of PRP and guided tissue regeneration in bone defects on dental implant stability with early insertion.*

*An experimental study has been done on 10 experimental dogs. 40 BCT implants were inserted, 4 for each dog (two on the left side and two on the right side, with guided tissue regeneration). Mobility of the implants was measured with the Periotest tool, first immediately after insertion and then 10 weeks after insertion. Results of measuring implant mobility according to the Periotest scale showed that all four groups of implants can be classified as belonging to the 0 degree implant mobility group.*

*On the basis of the results we conclude that the mobility of dental implants was lowest in the protocol where PRP was applied combined with bovine deproteinized bone and resorbable membrane of bovine origin.*

*Key words: dogs, dental implant, PRP, guided tissue regeneration, early implant insertion*

**INTRODUCTION**

The main objective of implant insertion is to achieve a structural and functional integration of the implant in the site where it is inserted keeping aesthetic criteria at the same time. The process in establishing the structural and functional integration of the implant is osseointegration. Osseointegration is a morphological and functional relationship between bone and the surface of a dental implant. This physiological state can be formed and maintained only if the

implantologist is fully aware of basic biological principles including the fact that bone is dynamic tissue, an organ, and a system. This should also include understanding basic principles of cell damaging, inflammation, bone healing, bone formation and remodelling, as well as physical properties of the implant and its surface. Failing to understand these principles leads to a failure in osseointegration. As a result, the implant is surrounded by dysfunctional fibrous tissue (Sumiya *et al.*, 1989).

Insertion of endosseous implants directly in the extraction alveolus was first introduced in the work of Schulte *et al.* (1979). Today, it is one of the most important alternatives in treatment of edentulouism. There are several different classifications of protocols for inserting dental implants in the extraction alveolus. A more clear definition of types of dental implant insertion following tooth extraction should be based on morphological and histological changes that follow tooth extraction. According to Hammerle *et al.* (2004) there are four protocols for placing implants in the extraction alveolus.

- Implant insertion in the alveolus immediately after tooth extraction (immediate insertion);
- Implant insertion after covering the alveolus with soft tissue (typically 4-8 weeks after extraction);
- Implant insertion in the alveolus after it has been considerably filled with bone tissue, which typically happens 12-16 weeks after tooth extraction;
- Implant insertion in the extraction alveolus with both bone and soft tissue having completely healed; this is usually the case 16 weeks after extraction.

Basic preconditions for successful bone healing are the same in both immediate and delayed implant insertion, regardless of whether it is insertion into the extraction alveolus or into an already healed alveolar ridge. It is important to note that, in the case of insertion into the extraction alveolus, there is a certain gap between the surface of an implant and the alveolar wall. This gap has to be filled with bone tissue so that osseointegration can take place. Bone healing depends on the stability of the initially formed coagulum in this area. Experimental studies on animals showed that critical factors in the stabilization of the coagulum are the distance between the bone and the implant and characteristics of the implant surface (Knox *et al.*, 1991; Stentz *et al.*, 1997; Akimoto *et al.*, 1999; Botticelli *et al.*, 2003). The absence of intact alveolar walls can have a negative influence on the stability of the blood coagulum and bone formation. To achieve bone regeneration and implant osseointegration in this case a technique of guided tissue regeneration should be applied (Gher *et al.*, 1994; Akimoto *et al.*, 1999).

Guided tissue regeneration in implantology is a method based on separating bone from soft tissues using membranes, which creates a gap that is to be filled with new bone tissue that is formed (Hockers, 1999). Numerous researches showed its efficacy in achieving filling up of bone defects which surround dental implants (Dahlin *et al.*, 1991; 1995; Buser *et al.*, 1990; Jovanovic *et al.*, 1992).

Platelet-rich plasma (PRP) is a concentrate of platelets in a small volume of plasma which can be produced of the patient's blood with a centrifuge in a blood bank, or in a preoperative process in a dentist's surgery. Growth factors that can

be found in the granules of platelets are a part of PRP. They are released when the platelets are activated by initiating healing (Marx *et al.* 1998.; Marx *et al.*, 2005; Anitua, 1999; Sonnleitner *et al.*, 2000). Since it is simple for use and easily available, plasma rich with concentrated platelets has become the most frequently used source of growth factors in implantology in the last two years, aiming at establishing as a wide contact surface between bone and implant as possible, which provides:

- improved stability of the implant,
- functional loading of the implant in a shorter time.

Growth factors that PRP contains relevant for bone regeneration are:

- 1) platelet derived growth factor (PDGF), and
- 2) transforming growth factor (TGF-beta).

The aim of the research was to clinically test the influence that PRP and guided tissue regeneration in bone defects have on dental implant stability with early insertion.

#### MATERIALS AND METHODS

The experimental research was conducted in three phases. It was done on 10 adult dogs, following all ethical principles imposed by the MMI act no. 282-12 issued on 02/11/2002.

##### *First phase of the experiment*

Doses of 0.03 mL/kg of Combelen intravenously and 0.01 mg/kg of body mass of atropine subcutaneously were used as pre-medication. Fifteen minutes after pre-medication a dose of 0.3 mL/kg of body mass of Ketamine chloride 5% was injected intramuscularly.

During the short intravenous anaesthesia the third and the fourth premolar on the both sides of the lower jaw were extracted. The extraction was done so that dental implants could be inserted in the positions of the third and the fourth premolar once the extraction injuries have healed. The extraction wounds were closed with a separate surgical closure (Dexon 3.0, Davis & Gack). A period of 8 weeks is considered to be sufficient for bone healing to take place, since the rate of bone healing for dogs is somewhat higher than for the human population.

All experimental animals were given intravenously an antibiotic (Jugocilin 1600000 i.u.). 12 hours after extraction the dogs were given food and water. They were fed with stew and kept in specially made boxes of prochrome that are easy to keep clean and disinfected. The dogs were housed at the Institute for Medical Research with conditioned air humidity and temperature of 21°C.

##### *Second phase of the experiment*

8 weeks after the extraction of the third and the fourth premolar on both sides of the lower jaw. Blood samples were taken from the animals using 3 sterile test tubes for preparing PRP (The method of making PRP was taken from Sonnleitner D).

### *Implant insertion*

The second phase of the experiment included early insertion of implants. Artificial per-implant bone defects were made around the implants' neck on the medial side of the implant. The horizontal size of the bone defects was 2 mm and their depth was 4 mm. A cylindrical BCT implant was placed into the extraction alveoli. The implant had 5 threads, a SLA surface, overall length of the neck and body was 17.5 mm, radius 4.5 mm in the neck and 3 mm in the body. The distance between the threads was 1.75 mm.

Immediately after placing the implant in the site the bone defects were filled, following the relevant protocol and using platelet-rich plasma produced from each animal one hour before the surgical intervention. A scheme of the arrangement of the inserted implants and filling of the bone defects is given in Figure 1.

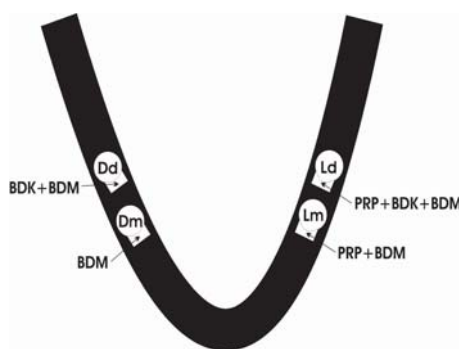


Figure 1. Scheme of the arrangement of the inserted implants and filling of the bone defects. Dd – right distal; Dm – right medial; Ld – left distal; Lm – left medial. BDK – bovine deproteinized bone; BDM – bovine demineralized membrane; PRP – platelet-rich plasma

Guided bone regeneration was used for filling in the bone defects. It was done in the following way:

- With platelet-rich plasma (PRP) combined with bovine deproteinized bone (BDK) and resorbable 150  $\mu$  thick membrane of bovine origin on the left side of the lower jaw in the extraction alveolus of the third premolar (Ld – left distal).
- With bovine deproteinized bone (BDK) and resorbable 150  $\mu$  thick membrane of bovine origin (BDM) on the right side of the lower jaw in the extraction alveolus of the third premolar (Dd – right distal).
- With platelet-rich plasma (PRP) and resorbable 150  $\mu$  thick membrane of bovine origin (BDM) on the left side of the lower jaw in the extraction alveolus of the second premolar (Lm – left medial).
- With resorbable 150  $\mu$  thick membrane with bovine origin (BDM) on the right side of the lower jaw in the extraction alveolus of the second premolar (Dm, right medial).

This was followed by measuring initial stability of the inserted implants using Periotest.

### *Measuring implant mobility using Periotest*

Immediately after placing the implants into their sites their mobility was measured. This was done using Periotest (Simens, Figure 2) with the standard scale of the Periotest values.

Table 1. Scale of the Periotest values

Mobility degree 0	-08 - +09
Mobility degree 1	+10 - +19
Mobility degree 2	+20 - +29
Mobility degree 3	+30 - +50



Figure 2. Measuring tool Implant mobility – Periotest (Simens)

A transepithelial extension was placed on the implant connection, which enabled appropriate measuring of implant mobility. The probe for measuring mobility had to be at a distance of 0.5-2.5 mm from the object that was measured. The angle between the probe and the axis of the measured implant was 90°. Measuring was done three times for each implant. Once measuring was completed, a security screw was placed for the resting period. The implants were covered with a mucoperiosteal flap which was attached with separate closures.

*The third phase of the experiment*

Ten weeks after implant insertion in the lower jaw the animals were put under anaesthesia in the same way as described for the first phase of the experiment. Measuring implant mobility was done in the same way as described for the second phase of the experiment.

## RESULTS

Table 2 contains main descriptors of separate samples with respect to dental implant stability in relation with the protocol was applied. Two teeth were extracted, II and III premolar, from the lower jaw of each dog followed by early insertion of dental implants. On each of the separate samples (size  $n = 10$ ) one of the described protocols of guided bone regeneration was applied.

Table 2. Final mobility of PTV implants (10 weeks after insertion)

Protokol GBR	No of observations	Mean (%)	Standard deviation	95% confidence interval		Min.	Max.
				Lower limit	Upper limit		
1 - PRP+BDK+RBDM	10	-6.96660	.760990	-7.51098	-6.42222	-8.000	-5.333
2 - BDK+RBDM	10	-6.93340	.378444	-7.20412	-6.66268	-7.667	-6.333
3 - PRP+RBDM	10	-6.83340	.477933	-7.17529	-6.49151	-7.667	-6.000
4 - RBDM	10	-5.06670	4.020779	-7.94299	-2.19041	-7.333	5.333
Total	40	-6.45002	2.146358	-7.13646	-5.76359	-8.000	5.333

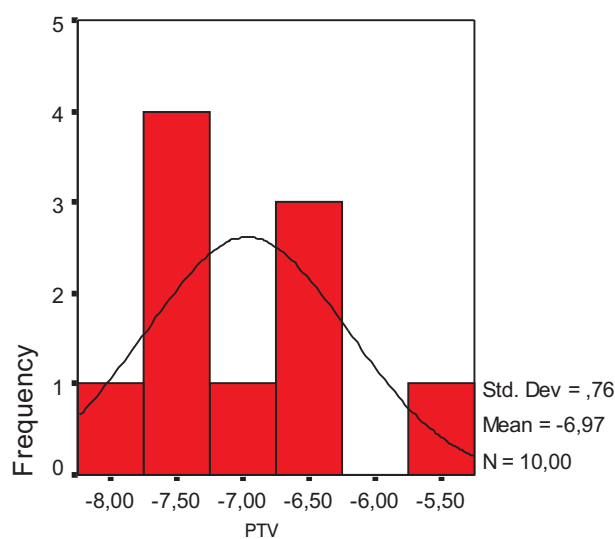


Figure 3. Frequency of measured PTV of protocol I – PRP + BDK + RBDM

The graphs show histograms of the frequencies of the measured PTV for different protocols and basic descriptors of separate samples (arithmetic mean, standard deviation, and N – the number of bone defects in the sample).

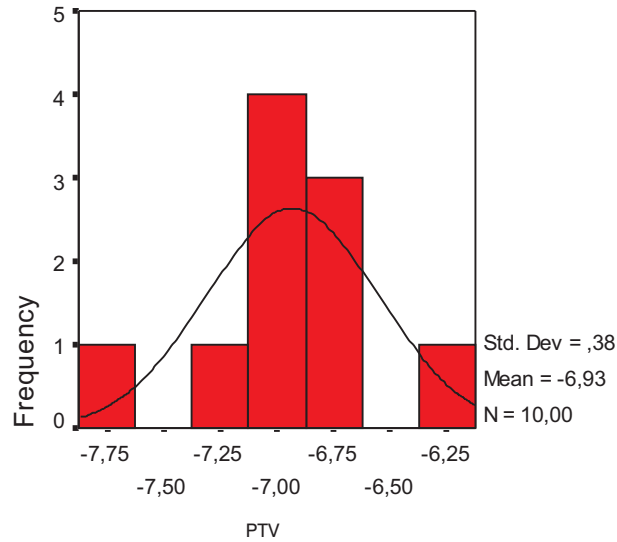


Figure 4. Frequency of measured PTV of protocol II – BDK + RBDM

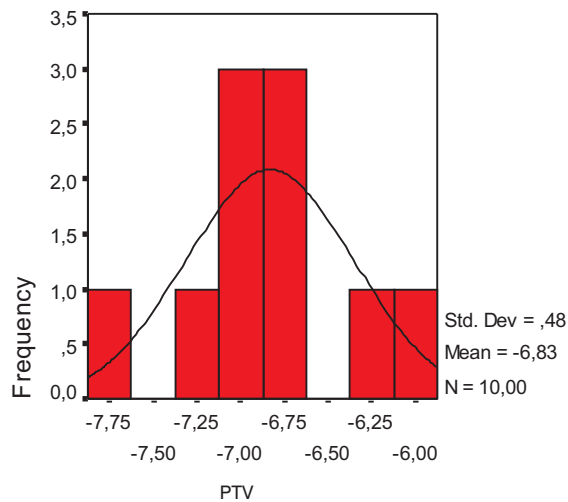


Figure 5. Frequency of measured PTV of protocol III – PRP + RBDM

Measurements were done using Periotest. The most negative values: arithmetic mean 7.97, the lower and the upper limit in the confidence interval (95%), as well as the interval of variation (-8 to -5.333 PTV) were recorded with

group "I". It means that this method provides better results than the others with respect to stability in early implant insertion. On the contrary, the worst results were recorded respectively with the sample that was treated according to the protocol "IV" – RBDM.

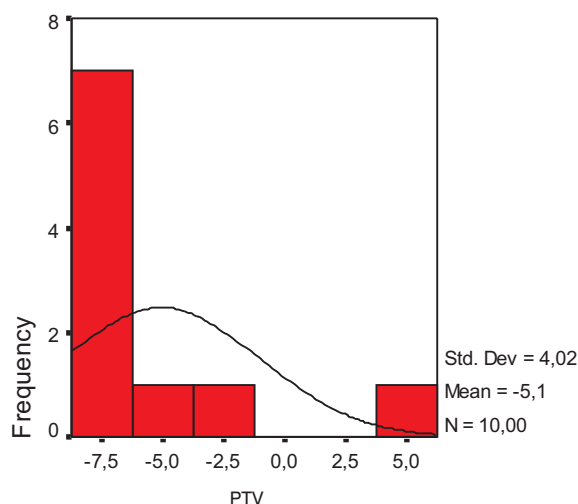


Figure 6. Frequency of measured PTV of protocol IV – RBDM

To see if there is a significant difference between the results of measuring implant mobility 10 weeks after insertion for the four applied protocols of guided bone regeneration, we applied analysis of variance (ANOVA).

Table 3. ANOVA – implant mobility 10 weeks after insertion

Variance	Square sum	Degree of freedom (df)	Square sum mean	F $V_F/V_R$	Sig.
Between groups ( $V_F$ )	25.611	3	8.537	1.995	.132
Intra groups ( $V_R$ )	154.057	36	4.279		
Total variance ( $V_T$ )	179.667	39			

F score,  $F = V_F/V_R$ ,  $F = 1.995$ . The score is higher than 1 which is in accord with the claim that the applied protocols differ with respect to their influence on implant stability. However, F score was lower than the critical value in the F score table of values. For a significance level of 0.05, with the degree of freedom of factor variance  $v_1 = 3$  and the degree of freedom of residual variance  $v_2 = 36$  the critical value was  $F_{\alpha; v_1, v_2} = F_{0.05; 3, 36} = 2.86$ . Hence,  $F < F_{\alpha; v_1, v_2} \Leftrightarrow 1.995 < 2.86$ . With a level of significance of 0.132 there is no basis for rejecting the null hypothesis that the



arithmetic means of all the sets are equal. In other words, we adopted the claim that there is no difference in dental implant stability depending on the applied protocol.

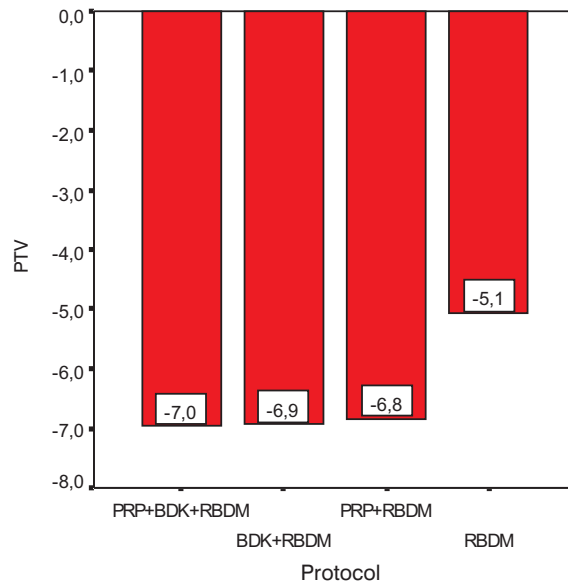


Figure 7. Mean values for implant mobility for each protocol

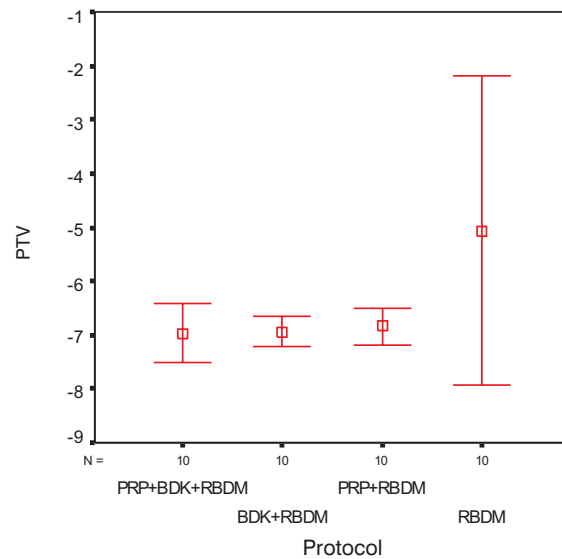


Figure 8. Dispersion of the PTV values close to the mean values for each protocol

We can see on the graphs of mean values and dispersion of values, in CI=95%, i. e.  $\pm 2SD$ , that protocols I, II, and III have similar mean values and that they are more homogenous than protocol IV. This indicates smaller oscillations in implant mobility when these protocols are used. However, it should be noted that the mean values for all the protocols belong to the most quality group of implant stability – the "zero" group.

#### DISCUSSION

Increased implant mobility is a direct indicator of inadequate osseointegration. Lack of bone tissue in oral implantation can make supported prostheses and implant treatment impossible. This is why the mobility test is a specific diagnostic method of detecting lack of osseointegration and a key factor in making a decision whether to remove the involved implant or not. Periotest values are a reliable indicator of the mobility of dental implants. These values can serve as a basis for assessing the relation between an implant and bone (Olive *et al.*, 1990).

The work of Mericske-Stern *et al.* (1995) pointed out the negative Periotest values that can occur after 3 months of healing (Periotest values -1 to -8), with similar results occurring 1 year after implant loading (Periotest values -2 to -8).

Assessing implant mobility immediately after insertion and 3 months after immediate insertion into the extraction alveolus with dogs shows that high values of stability were achieved in all cases and all implants belong to the "zero" mobility group (mobility -8 to +9) and that, according to this, all the protocols provide satisfying results. Examination of statistical significance of the differences between the mean values of implant mobility 3 months after immediate insertion into the extraction alveolus according to the four protocols of guided bone regeneration showed that there is no significant difference between the protocols. Nevertheless, the best results were recorded with protocol I (PRP-BDK-RBDM, platelet-rich plasma + bovine deproteinized bone + resorbable membrane with bovine origin). The worst results of stability of dental implants are recorded with the sample where protocol IV (RBDM, resorbable membrane with bovine origin) was applied. Cune *et al.* (1996) point out that Periotest values for osseointegrated implants should not exceed the value of +3 and should be negative.

Barber *et al.* (1996) used Periotest to compare osseointegrated implants with and without healing abutments. The Periotest measurements were done immediately after insertion and 5 months after insertion. The results show that there is a big difference in the Periotest values (PTV) for implants immediately after insertion in comparison with implants 5 months after insertion. The values immediately after insertion are higher, in the range -0.7 to -0.4. The values 5 months after insertion are within the range -0.5 to -0.3. All the 50 Periotest values in this study were negative, which means decreasing mobility. The lower values for the measurement after 5 months can be explained by bone maturation, which reduces the negativity of PTV values.

Rotter *et al.* (1996) used Periotest to examine patients with 26 implants, 15 of which were loaded progressively and 11 were left without load until final

prosthetic restoration. All PTV in this study were about 0 index, meaning that there was no implant mobility. However, the value were different and an increased rigidity (higher negative values) could be noted with the implant that were progressively loaded. These results brought to the conclusion that submitting the implant to a limited force during the healing period can have positive effects on bone healing and extent of osseointegration.

Research of Ericsson *et al.* (1997) showed that, measured 5 years after insertion, implant stability is equal in both cases – with either one step and two step insertion techniques. PTV values were negative in both cases, ranging from -6 to +3 in the case of one step technique, while the interval was a little wider for the two step technique.

Simunek *et al.* (2002) compared the stability of titanium implants and implants made of titanium and coated with hydroxyapatite. Using Periotest they got PTV values that indicate a more rigid connection of the hydroxyapatite-coated implants than it is the case of implants with a titanium surface. The PTV values were within the range -8 to +2 for both types of implants in the initial phase, with no statistical difference between the mean values for the two groups. One year after insertion the values were -2.30 for the hydroxyapatite-coated implants and -1.89 for the other group.

Using Periotest in measuring implant mobility in "*in-vitro*" conditions proved that it is a reliable indicator of bone loss around an implant (Lachmann *et al.*, 2006). However, Lachmann *et al.*, (2006) keep some reserve in their conclusions, arguing that one should never rely on just one method in clinical testing.

On the basis of our research can be concluded that dental implant mobility is lowest for the protocol where platelet-rich plasma was used combined with bovine deproteinized bone and resorbable membrane of bovine origin.

Future research should be directed towards identifying different growth factors, examining their influence, and defining their optimal concentration in different protocols of dental implant insertion.

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## EFEKAT LOKALNE PRIMENE PLAZME BOGATE TROMBOCITIMA I VOĐENE TKIVNE REGENERACIJE NA STABILNOST DENTALNIH IMPLANTATA

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### SADRŽAJ

Oseointegracija dentalnih implantata nastaje iz ćelijske migracije, diferencijacije, koštane formacije i koštane remodelacije duž površine implantata; svaki od ovih procesa je zavistan od trombocita i krvnog koagulumata. Plazma bogata trombocitima (PRP) se primenjuje da bi se povećala oseointegracija odnosno stabilnost implantata. To je frakcija krvi dobijena centrifugiranjem koja sadrži visoke koncentracije trombocita. Trombociti sadrže brojne faktore rasta poput PDGF, TGF- $\beta$ , IGF, VEGF i druge koji doprinose tkivnim reakcijama. Cilj rada je bio da se klinički ispita uticaj plazme bogate trombocitima i vođene tkivne regeneracije u koštanim defektima kod rane ugradnje dentalnih implantata na pokretljivost dentalnih implantata. Eksperimentalna studija je sprovedena na 10 eksperimentalnih pasa kod kojih je ugrađeno 40 BCT implantata i to po 4 (dva sa leve strane i dva sa desne strane uz vođenu tkivnu regeneraciju). Merenje pokretljivosti implantata vršeno je neposredno nakon ugradnje i 10 nedelja nakon ugradnje aparatom Periotest. Rezultati merenja pokretljivosti implantata prema Periotestnim vrednostima su pokazali da se ugrađeni implantati u sve 4 grupe mogu svrstati u grupu 0 stepena pokretljivosti.

Na osnovu dobijenih rezultata došli smo do zaključka da je pokretljivost dentalnih implantata najmanja u protokolu gde je primenjena plazma bogata trombocitima u kombinaciji sa bovinom deproteinizovanom kosti i resorptivnom membranom bovinog porekla.

