Bone Conditioning to Enhance Implant Osseointegration: An Experimental Study in Pigs

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Purpose: *Osseointegration of implants depends on time and the local bone conditions regarding quality and quantity. This led to the bone classification by Lekholm and Zarb. The aim of the present study was to enhance osseointegration of implants through conditioning of the bone bed and to compare in this context the efficacy of bone condensation, an osteoinductive collagen (Colloss), and platelet-rich plasma (PRP).* ***Materials and Methods:*** *Porcine frontal skull bone was used for the preparation of identical-size implant beds. Before placement of the implants (Ankylos, 3.5×4 mm), the implant beds were untreated (control) or conditioned with condensation, Colloss, or PRP. The animals were sacrificed after 2, 4, and 8 weeks. The specimens were then compared and analyzed by microradiography, and statistical analysis was performed using the Wilcoxon signed rank test.* ***Results:*** *At the early observation times, significant effects on the sites of topical bone conditioning in comparison to the control group could be seen regarding the implant-bone interface (2 weeks: control 31%, Colloss 60%, condensation 73%, PRP 47%; 4 weeks: control 39%, Colloss 51%, condensation 40%, PRP 42%) and peri-implant bone density (2 weeks: control 31%, Colloss 48%, condensation 59%, PRP 39%; 4 weeks: control 47%, Colloss 53%, condensation 41%, PRP 50%). A leveling of the results between groups was found at 8 weeks (implant-bone interface: control 51%, Colloss 58%, condensation 55%, PRP 62%; peri-implant bone density: control 50%, Colloss 55%, condensation 51%, PRP 51%).* ***Discussion:*** *Overall, bone condensation and Colloss apparently influenced bone formation process from the onset, but over the entire 8-week healing period, differences in bone formation were not significant.* ***Conclusion:*** *It can be stated that, in the initial healing phase, an effect of topical bone conditioning may be achieved by the different described methods.* (INT J ORAL MAXILLOFAC IMPLANTS 2003;18:505–511)

Key words: *bone condensation, collagen, dental implants, osseointegration, platelet-rich plasma*

An important clinical factor for the success of endosseous implants is their rate of osseointegration. Clinical surveys of the past decades have shown significant differences for the survival rates of implants in relation to their localization.[1,2] Possible criteria for these differences include topical differences in the histologic bone morphologies of the maxilla and mandible.[3,4] While in the mandible Class 1 or 2 bone quality can be found, the maxilla generally presents with Class 3 or 4 bone quality according to Lekholm and Zarb.[3] Radiation has been described as an additional negative influence on prognosis.[5,6] This is especially important when implants are placed in patients who have undergone radiation for treatment of neoplasms. In these cases, not only does the topical condition of the implant bed worsen the prognosis, but the reduced quantity of bone often necessitates the use of extremely short implants.[6–8] The purpose of this experimental study was to evaluate microradiographically the outcome of implants placed in bone that was given different topical treatments.

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Fig 1a Clinical image of the circular coverage of the implant with Colloss.

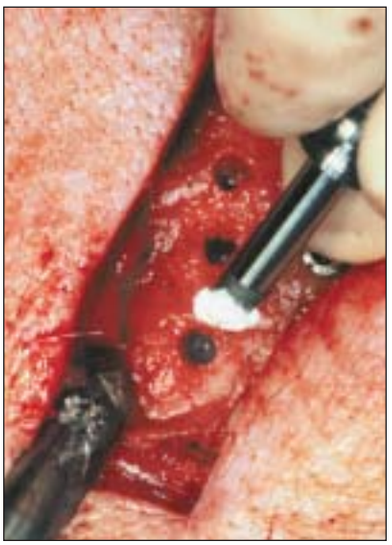


Fig 1b *(Right)* Placement of the Colloss-covered implant into the prepared implant bed.

MATERIALS AND METHODS

Nine adult pigs, each 12 months of age, were included in the study. The research project was approved by the Animal Research Committee for the government of Midfrankonia (approval no. 621-2531.31-5/00), Ansbach, Germany. For all surgical interventions, the animals were anesthetized by an intravenous injection of ketamine hydrochloride (Ketavet; Ratiopharm, Ulm, Germany). After local anesthesia was obtained (Ultracain DS forte; Hoechst, Frankfurt, Germany) in the area of the frontal skull, a sagittal incision was made and the soft tissues and periosteum were mobilized. The frontal skull of the animals was chosen because of the following properties: *(1)* it provides comparable placement sites inter- and intraindividually; *(2)* the structure of the bone under consideration is of desmogenous origin and not vascularized by a central blood vessel; and *(3)* the bone quality is Class 2 or 3.[9] Each pig received a total of 16 implants (n = 144 total). The implants were randomly assigned to 4 groups of 4 implants placed in a sagittal row from left to right.

In group 1, placement of the implants was executed according to the guidelines of the manufacturer without any additional measures; this group served as the control. In group 2, the implant surface was covered with bovine collagen (20 mg Colloss; Ossacur Medical Products, Oberstenfeld, Germany) and then placed in the prepared implant beds (Figs 1a and 1b). Colloss is a collagen of bovine origin, which because of its properties, leads to local adhesion and aggregation of thrombocytes.[10–12] In group 3, initially harvested blood from the jugular vein was prepared according to the method described by Marx and coworkers[13] and modified according to the guidelines of Curasan Pharma (Kleinostheim, Germany). The preparation led to an average increase in the number of thrombocytes of 5.0 compared to the initial counting.[14] One half milliliter of this platelet-rich plasma (PRP) was placed into the implant beds and 0.25 mL was applied directly to the surface of the implants before placement in the implant bed (Fig 2). For group 4, only the initial spiral bur (2.0 mm diameter) was used for the preparation of the implant bed. Then the bone bed was further widened using bone condensers (Stoma Instruments, Tuttlingen, Germany) in an ascending row to finalize preparation of the implant bed by the lateralization of bone[15–17] (Fig 3).

The soft tissues were readapted and the wounds were closed by resorbable sutures (Vicryl 2.0; Ethicon, Norderstedt, Germany). Anesthesia was then terminated by injection of Revivon (Pherrovet, Malmö, Sweden). For the first 3 postoperative days the animals received streptomycin (0.5 g/day; Grünenthal, Stolberg, Germany).

In the postoperative period, the animals were marked with Ca+-affine markers (rolitetracycline 12 mg/kg, Merck, Darmstadt, Germany; xylenol orange 30 mg/kg, Fluka, Taufkirchen, Germany; alizarin 30 mg/kg, Serva, Heidelberg, Germany; and calcein green 20 mg/kg, Fluka) according to schedule. Animals were sacrificed by an overdose of pentobarbital (Dermocal, Buenos Aires, Argentina) after 2, 4, or 8 weeks. The os frontale was harvested and the specimens were fixed by immersion in formalin solution, dehydrated in alcohol, and embedded in acrylic resin for histologic examination by means of undecalcified

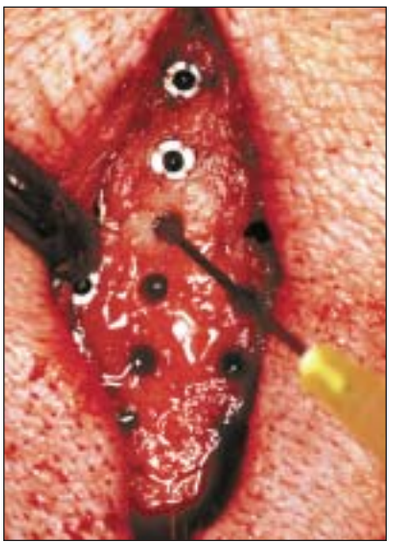
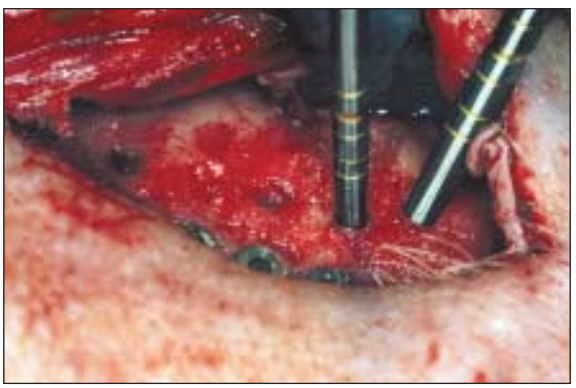


Fig 2 *(Left)* Topical application of the PRP prior to implant placement.

Fig 3 *(Below)* Topical bone conditioning by lateral condensation.



sections using the technique described by Donath and Breuner.[18] Undecalcified sections 180 µm thick were produced in a mesiodistal direction parallel to the long axes of the implants. These histologic specimens were subjected to microradiographic analysis (Faxitron; Rohde and Schwarz, Cologne, Germany) and planimetrically analyzed as described by Matsui and associates.[19] Statistical analysis was done with SPSS software (Chicago, IL) using the Wilcoxon signed rank test ($P < .05$).

RESULTS

Bone-implant Interface

At 2 weeks, the control group had a bone-implant interface of 31%; all test groups showed significantly higher values. Group 4 samples (bone condensation) reached the maximal value of 73% (Fig 4a), the Colloss group achieved 60%, and the PRP group reached 47% (Fig 4b).

Four weeks after placement, the control group had a 39% bone-implant interface; the Colloss group reached 60% (Fig 5), the PRP group reached 42%, and the bone condensation samples showed a decline from the 2-week group, to 40% (Fig 6).

At the final observation time of 8 weeks, a leveling of the results could be seen. The control group reached 51%, the PRP group 62%, the Colloss group 58%, and the bone condensation group 55% (Figs 7a and 7b).

Peri-implant Bone Density

At the first investigation period of 2 weeks, the control group achieved a peri-implant bone density of 31%, the bone condensation group achieved 59%, the Colloss samples reached 48%, and the PRP group reached 39% (Fig 8).

Similar to the values found for the implant-bone interface at 4 weeks, for bone density at 4 weeks a significant decline in the bone condensation samples was seen (41%), versus 47% in the control group. The Colloss group gained density, to 53%, whereas the PRP group showed an increase of over 10%, to 50% (Fig 9).

Similar to the implant-bone interface findings, a leveling of the peri-implant bone density was evident at 8 weeks. The control group reached 50%, the bone condensation group 51%, the PRP group 51%, and the Colloss group 55% (Figs 10a and 10b).

DISCUSSION

Overall, bone condensation and Colloss apparently influenced the bone formation process from the onset. This could be the explanation for the high bone density measured by microradiography in these groups at 2 weeks. By the technique of bone condensation, a lateralization of microscopic fragments of fractured bone was achieved, leading to the initially high values in this group. For PRP as the third test group, the results were inferior, but not remarkable if one considers the biokinetics of PRP. As is commonly known, PRP develops its activity peak within the first week after application, but these biochemical reactions are only the initiating procedures that finally lead to de novo bone formation and amplification of density in the microradiography by mineralization.[20,21] The physical bone

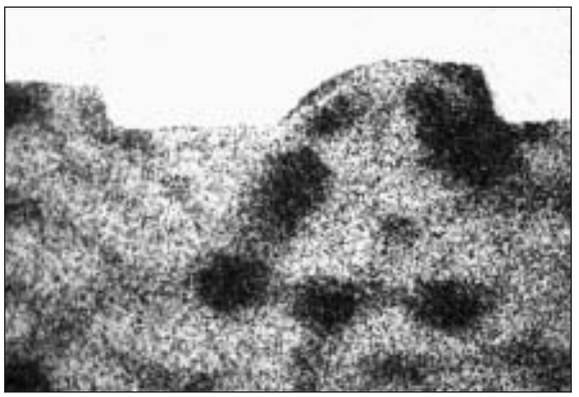


Fig 4a Implant-bone interface at 2 weeks (sample treated using condensation technique), as observed through the Faxitron system.

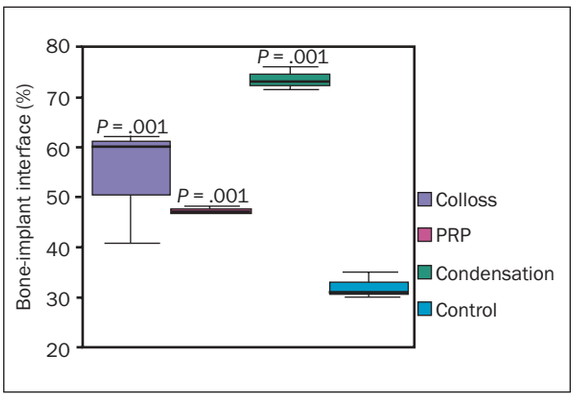


Fig 4b Box plot of the implant-bone interface at 2 weeks. Significant differences were found in all 3 test groups in comparison to the control group.

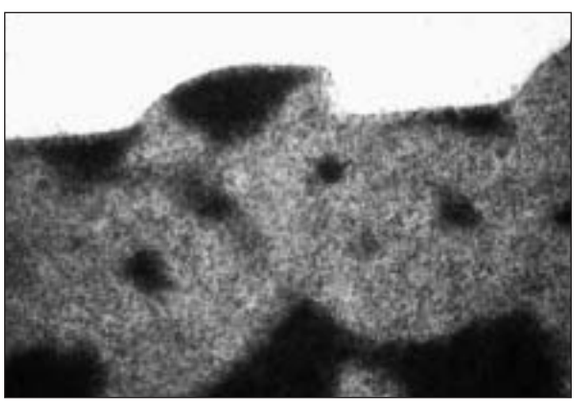


Fig 5 Implant-bone interface at 4 weeks (Colloss). A significantly greater increase was found in the Colloss group versus the control group, but a decrease was seen in the condensation group.

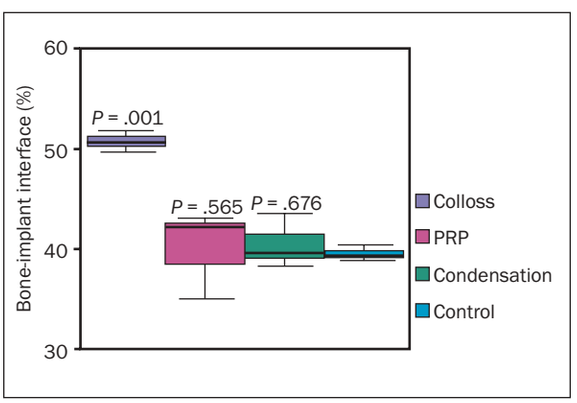


Fig 6 No significant differences in bone-implant interface percentage were seen between the groups at 4 weeks. Significant differences were only seen in the Colloss group at 4 weeks.

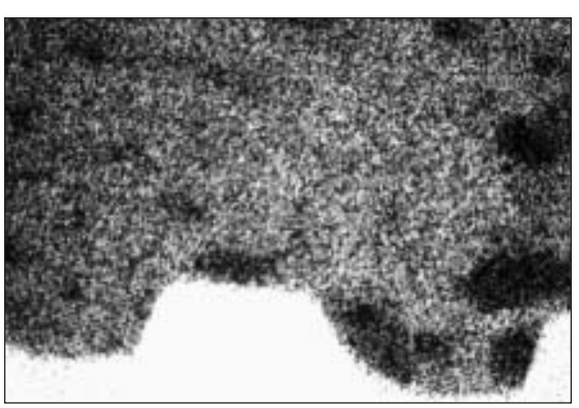


Fig 7a Implant-bone interface at 8 weeks after condensation.

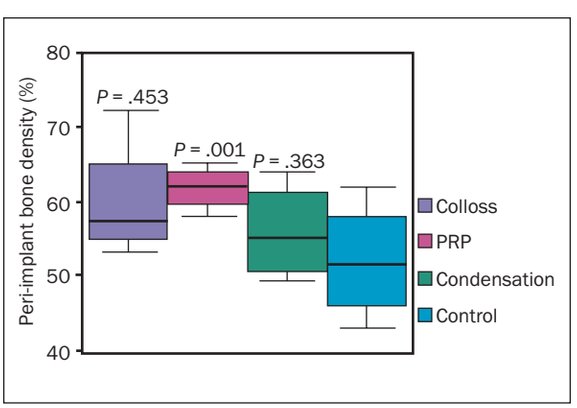


Fig 7b Box plot of bone-implant interface at 8 weeks, showing significantly higher values in all 3 test groups. The values found for mechanical condensation were 10% higher than for the other techniques.

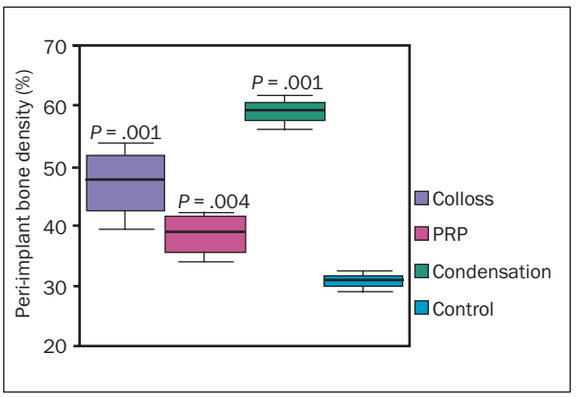


Fig 8 A dramatic increase in bone density was seen at 2 weeks using the condensation technique.

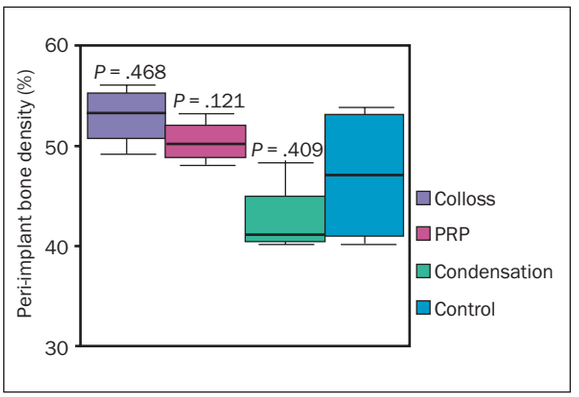


Fig 9 No significant difference in bone density values was seen in the test groups versus the control at 4 weeks.

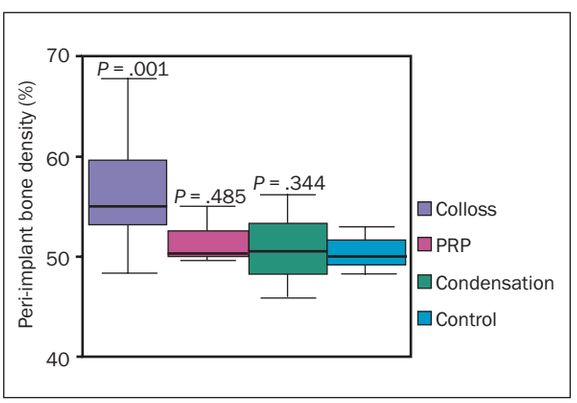


Fig 10a At 8 weeks a leveling off of the peri-implant bone density was observed; nevertheless, the Colloss-treated group showed significantly higher values.

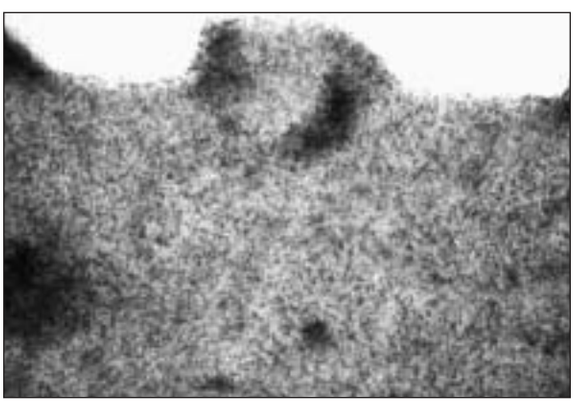


Fig 10b Bone density observed at 8 weeks in a sample treated with Colloss.

condensation and higher initial density related to the combined placement of implants with Colloss are not necessarily convincing results of an initial bioreaction of the bone, but may be explained as results of mechanical effects: On day 1, the sponge-like Colloss mass is pressed into the trabecular spaces around the bone bed.

By the biochemical result of the bone drilling alone, the opening of trabecular spaces may release autogenous bone morphogenetic proteins (BMPs) and initiate the complete cascade of a healing process.[22] Topical PRP from the bone bed itself will likely influence the wound area in addition to the inserted PRP.[20–24] But any effect of PRP probably is limited to less than 1 week.[20,24,25]

Subsequently, the platetelet-derived growth factors (PDGF) expressed by macrophages dominate bone healing. Because of the hypoxide and acid situation within the non-vascularized dead space of the bone wound, macrophages are attracted and begin osteoclastic activities.[26] The PRP-fixed proteins, enzymes, and peptides provide for the induction of a specific cell response to control the tissue-implant interface with molecules delivered to this interface. The adhesion molecules are mediators of the attachment of cells to extracellular matrix proteins such as collagen Type I, osteopontin, or fibronectin.[22,26–29] By delivery of these molecules directly to the tissue-implant interface, it is possible to promote bone formation.[13,28,29]

There is a time lapse before this stimulated bone formation can start, as evidenced by comparing

cases in which bone condensation happened mechanically or under the influence of a hydrophilic collagen as Colloss. Colloss, if considered to have properties of the family of BMPs, has bone-inducing power without the dependence on topical bone responder cells like osteoblasts.

BMPs are compounds that induce new bone formation because of a morphogenetic quality to modify mesenchymal tissue at the site of implantation.[21,30–36] Growth factors and cytokines like PRP, in contrast, change the growth rate of pre-existing bone.[13,20,22,24–26] This hypothesis is the basis for application of PRP directly into the prepared implant bed to promote osseointegration.[13] PRP contains all of the important growth factors but needs osteoblasts or progenitor cells to be effective in bone formation. The osteoblastic activity will be enhanced by PRP, and osteoclastic resorption will be inhibited. Since a systemic effect is largely time limited as a result of the 50% PDGF clearing the blood stream after 2 minutes, the conclusion can be made that PRP is only topically effective, in contrast to BMPs.[20,25] The considered BMP properties of Colloss may explain the rapid increase in bone density around implants in these groups. In vitro studies seem to exclude a tumor-mitogenic effect of BMPs.[36] BMPs would thus act more as a differentiation factor than as a growth factor and thus might be expected to further differentiate tumor cells, rather than supplement their growth. In animal studies, bone formation created by BMP was already seen after 5 days.[36] No cartilage formation was visible; only direct bone formation was evident. Therefore, it may not be surprising that Colloss increases the bone density around the placed implants, both initially and continuing.

The topical bone condensation before implant placement is part of the creation of a regional acceleratory phenomenon (RAP), ie, a reaction of any noxigenic stimulus and promoter that results in tissue regeneration that is up to 10 times faster than normal.[27–29,37] RAP starts a few days after a bone lesion is set, achieves a peak after 1 month, and can persist in bone for 4 months or longer. The intensity of RAP is proportional to the intensity of the stimulus and dependent on the region.[28,29,37] During bone condensation, lateralization of infractured or fractured trabeculae leads to an immediate increase in density. The regionally initiated osteoclastic and osteoblastic activities that follow complete the primarily achieved bone press-density by intensive bone formation activity.

SUMMARY

Documented peri-implant osseoneogenesis occurred naturally in the controls, but was less impressive than that seen under the influence of Colloss, PRP, or bone condensation. Time provided for the increase in density under the influence of PRP. The increase of bone density in the control group, compared to the 2-week and 4-week results, was more impressive than that seen in the other groups. The approximation of bone density over the observation period demonstrates that the different bone bed conditioning methods can modulate the bone reaction initially, but after 4 weeks a leveling of density occurs. Clinically, it could be useful to employ any of the tested methods in cases where the bone bed is unfavorable or where previous diseases or treatment modalities have caused a negative bone reaction situation.

ACKNOWLEDGMENTS

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