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Enhanced periodontal regeneration using collagen, stem cells or growth factors

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TABLE OF CONTENTS

1. ABSTRACT

The regeneration of periodontal tissues still remains a challenge in periodontology. The aim of the present study was to examine the regenerative potential of a) different collagen support versus blank, b) different collagen support +/- a growth factor cocktail (GF) and c) a collagen powder versus collagen powder + periodontal ligament stem cells (PDLSCs) comparatively in a large animal model. The stem cells (SC) were isolated from extracted teeth of 15 adult miniature pigs. A total of 60 class II furcation defects were treated with the materials named above. Concluding, a histological evaluation followed. A significant increase in regeneration was observed in all treatment groups. The new attachment formation reached a maximum of 77 percent. In the control group a new attachment formation of 13 percent was observed. The study shows that all implanted materials improved periodontal regeneration, though there were no significant differences between the experimental groups. Within the limitations of this study, it can be assumed that the lack of significant differences is due to the complexity of the clinical setting.

2. INTRODUCTION

One of the most challenging goals in periodontics is the regeneration of the tooth supporting

structures, which are destroyed as a result of periodontal disease progression (1). Frequently the loss of teeth is caused by progressive bone resorption (2). Current periodontal surgical treatment options, like scaling and rootplaning or open-flap debridement, have been established to slow down the disease progression, but have been limited in periodontal regeneration (3). Complete regeneration seems to be the ultimate goal in periodontal research concerning the complexity of biological processes with a view to stem cells, growth factors and environmental conditions (1). Guided tissue regeneration (GTR) and osseous grafting are the most approved techniques having the greatest regenerative potential of surgical periodontal treatment so far (4,5). The reconstruction of alveolar bone by bone transplantation, bone substitute material and guided bone regeneration is daily clinical practice, but it is rather the formation of a long junctional epithelium and the insufficient formation of new cementum which makes periodontal regeneration challenging (6-8). A complete new periodontal apparatus is defined as the entirety of bone, cementum and periodontal ligament (Sharpey´s fibers) (2), to be referred to in the following as new attachment. The tooth disposes of a stable periodontal apparatus if the connection between the root surface and alveolar bone is composed of new generated periodontal ligament fibers inserted into new cementum (9).

The cells that are mostly reliable for the regeneration of cementum, alveolar bone and periodontal ligament (PDL) are cementoblasts, osteoblasts and fibroblasts (10). Precursors of these cells are mesenchymal stem cells, which can be differentiated according to their origin. Mesenchymal stem cells have the potential to differentiate into several cell types and can be cultured under defined tissue culture conditions *in vitro* (11). Various dental stem cells have been isolated and characterized: periodontal ligament stem cells (PDLSCs), dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth, stem cells from apical papilla and dental follicle precursor cells (12). PDLSCs were shown to have great potential in regenerative medicine (13). Due to the fact that stem cells need a suitable carrier material, a variety of different biomaterials is widely examined *in vitro* and *in vivo* (14). A lot of biomaterials can serve as carrier materials, such as natural or synthetic polymers, extracellular matrix, self-assembling systems, hydrogels or bioceramics. Scaffolds qualified for transporting stem cells are different types of collagen (15).

Growth factors in combination with biocompatible matrices play another important role in the regeneration of the periodontal ligament (16). At cellular level, wound healing and regeneration involve a complex cascade of events, including cell proliferation and differentiation. These processes are known to be modulated by growth factors (17). They stimulate cell proliferation *in vitro* and probably also *in vivo* (18).

The aim of the present study was to evaluate different regenerative approaches for periodontal regeneration in a large animal model, including the usage of stem cells and growth factors. Therefore, the regenerative effect of periodontal ligament stem cells and growth factors implanted on a collagen carrier was investigated in periodontal furcation defects in minipigs.

3. MATERIALS AND METHODS

3.1. Miniature pig animal model and ethical approval

Fifteen Göttinger minipigs (22 ± 3 months, 35 ± 11 kg), each exhibiting a fully erupted permanent dentition, were used for this study. The minipigs were housed under conventional conditions with free access to water. Diet had to be restricted according to age. The study protocol was approved by the appropriate local authority (German Decree on the Reporting of Laboratory Animals 7221.3-1.1.-075/11, Regional Authority for Agriculture, Food Safety and Fisheries, State of Mecklenburg-Western Pomerania, Germany). All surgical procedures were performed under anesthesia, and all efforts were made to minimize animal suffering.

3.2. Experimental workflow

The 1st and 2nd premolars of each animal were extracted on both sides of the lower jaw. The teeth (i.e., the periodontal ligament) were used for isolating and culturing stem cells. 90 days after tooth extraction class II furcation defects were created at the 3rd premolar and 1st molar in the mandible on both sides. The defects were randomly assigned with different treatment groups (see Phase 2: Implantation). Afterwards, all defects were covered with a semipermeable membrane. A polychrome sequential labeling was performed 14, 28 and 84 days after the replantation. The animals were sacrificed after 120 days of healing followed by a histological assessment (Figure 1).

3.3. Anesthesia

All surgical procedures were carried out under aseptic conditions and general anesthesia. Prior to each surgical intervention, the animals received an induction with ketamine 10 %, (Pfizer AG, NY, USA) plus 1.5. ml midazolam (Sanochemia Pharmazeutika AG, Neufeld, Österreich) by intramuscular injection. Intravenous access was established through ear vein and induction was continued by injection of 0.2.5 – 0.4. ml pancuronium (Organon Teknika, Eppelheim, Germany) for muscle relaxation. Following oral intubation anesthesia was continued by inhalation of isoflurane (AbbVie AG, Baar, Switzerland) and injection of 0.5 - 0.8. ml/min fentanyl (Janssen - Cilag, Neuss, Germany). The administration of oxygen was about 1,5 l/min.

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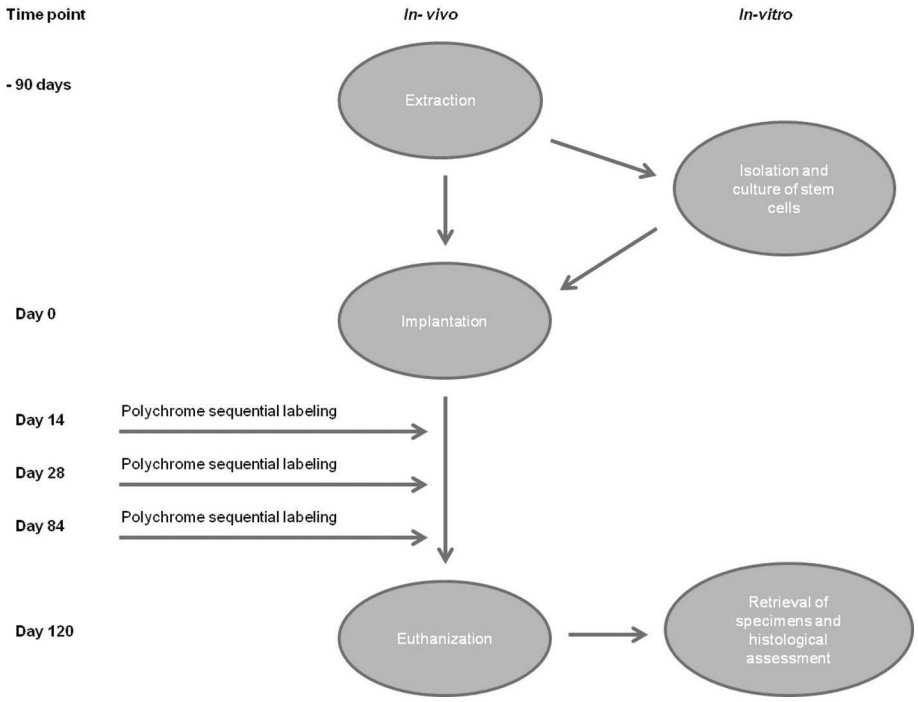

Figure 1. Experimental workflow. Within 210 **day**s the total procedure occurred, from extracting teeth over implantation to histological assessment of the specimens (see materials and methods).

After begin of general anaesthesia, the perioral hair was cut, the perioral tissues and the gingiva were disinfected with povidone iodine (Betaisodona ®, Mundipharma GmbH, Limburg an der Lahn, Germany) and the animals received a further local anesthesia (Ultracain D-S forte, 1:100 000, 2 ml, Sanofi-Aventis Deutschland GmbH, Germany). Intraoperative, antibiotics were administered as ampicillin/sulbactam 1000 mg/500 mg i.v. (HEXAL AG, Holzkirchen, Germany) intraoperative. For postoperative analgesia treatment the animals received a Metacam ® - suspension (15 mg/ml) with a dose of 2.7. ml/100 kg body weight and synulox 250 mg (Pfizer AG, NY, USA).

3.4. Surgical procedures

3.4.1. Phase 1: extractions

The 1st and 2nd premolars were extracted bilaterally in the mandible of the minipig. The periodontal ligament of the extracted teeth was obtained to isolate PDLSCs. All extracted teeth and viable materials were treated repeatedly with a rinsing solution, consisting of cooled phosphate buffered saline (PBS, 20 ml PBS 1x) and antibiotics (200 µl penicillin/streptomycin 1x, PenStrep, gibco, Grand Island, NY, USA) in order to reduce the risk of bacterial contamination. The samples were transported in DMEM-F12 (gibco, Grand Island, NY, USA) including 2 % antibiotics (PenStrep, gibco, Grand Island, NY, USA). They had to be kept on a constant temperature of + 4 °C.

3.4.2. Phase 2: implantations

In the second surgical intervention, class II furcation defects were created at the 3rd premolar and 1st molar in both quadrants of the mandible. Initially, a mucoperiosteal flap was formed by a gingival margin cut with a vertical relief cut mesial and raised with a raspatory. The defects were created with a bud burr and the bottom of the newly created defects marked with a notch for later histological evaluation.

The defects were randomly filled with the following materials: Group I - blank, Group II - collagen membrane, Group III - collagen membrane and growth factors, Group IV - collagen matrix, Group V - collagen matrix and growth factors, Group VI - collagen powder, Group VII - collagen powder and stem cells. With a sterile Heyman type spatula all materials were filled into the furcation defects. All defects were covered with a 35 X 45 mm semipermeable membrane (bredent medical, angiopore selective permeable membrane, Senden, Germany) and closed with a mucoperiosteal flap using absorbable sutures (ETHICON, VICRYL, 3-0, Polylactin 910, SH-1 plus) (Figures 2 and 3).

3.4.3. Phase 3: polychrome sequential labeling

The technique of polychrome sequential labeling is used in order to microscopically investigate the state of new bone formation and remodeling processes at different time intervals. Three different fluochromes were

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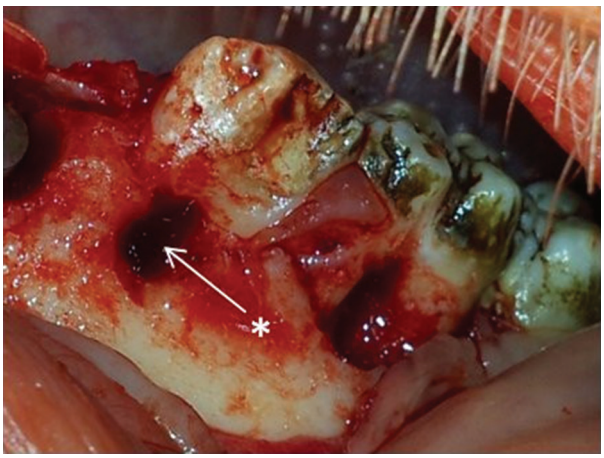

Figure 2. Class II furcation defect. This picture shows the 3rd quadrant of mandible while the mucoperiosteal flap is mobilized.

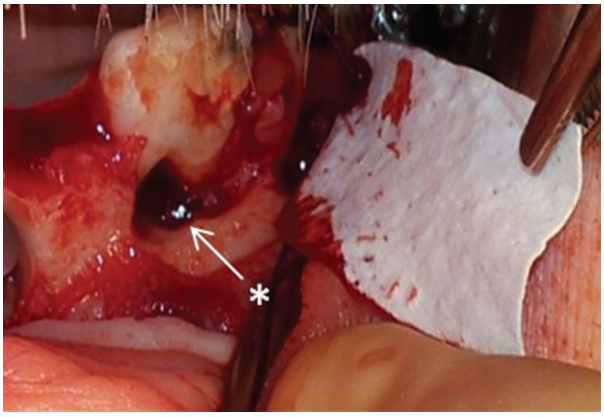

Figure 3. Class II furcation defect. This picture shows the semipermeable membrane before placing in the 3rd quadrant of mandible.

administered by intravenous injection 14, 28 and 84 days after implantation: xylenol orange (6 %, 2-5 g/animal), calcein green (1 %, 0.8.-1.5. g/animal) and alizarine complexone (3 %, 1-1.5. g/animal). Therefore, the animals were sedated by ketamin 10 %. Simultaneously, a clinical examination of the animals was performed.

3.5. Materials

Overall, 60 periodontal class II furcation defects were created surgically. Out of these 60 defects, three could not be evaluated because of a faulty manufacture of the specimens and another four were excluded from the study because of the presence of oral abscesses. The defects were treated with different materials. Several collagen materials were used as carrier materials.

First, a collagen membrane (DOT, Rostock, Germany), consisting of 1 % collagen mixed with hydroxylapatit/tricalcium phosphate (60:40) in mass relation of 4:1, was used. Also, a collagen matrix (Bioserv, Rostock, Germany) with collagen from jellyfish *Rhopilema spec.* was chosen. This biopolymer has natural extracellular matrix characteristics and low risks for infection (e.g. BSE). In addition, a collagen powder (MedSkin Solutions Dr. Suwelack AG, Billerbeck, Germany) made from bovine hide and containing fibrillar collagen type I, III and V was used.

The carrier materials were either implanted solely or combined with a growth factor cocktail or periodontal ligament stem cells. Earlier *in-vitro* tests showed that the stem cells could only be combined with the collagen powder, which had to be buffered to a suitable pH value with the help of a potassium buffer. When the stem cells were combined with the collagen membrane or collagen matrix, the 24 hours survival rate of the stem cells was too low.

A growth factor cocktail (DOT, Rostock, Germany) was also utilised in this study. The growth factors were dissolved in 0,9 % sodium chloride, resulting in a concentration of 2 mg/ml, transferred into 1 ml syringes. One syringe contained a mixture of VEGF, b-FGF, IGF-1 and TGF- ß1. Per defect 0,15 ml were used with an amount of 0,3 mg GF.

The periodontal ligament stem cells were isolated from extracted teeth and cultured according to Haddouti´s protocol with slight modification (19). For reimplantation a number of 10^6 cells of the expanded third passage was applied and the stem cells were implanted with the collagen powder. Therefore, the collagen powder was buffered to a suitable pH value with a potassium buffer and mixed with the stem cells with the help of a sterile pipette in a 24 wells micro plate. A spatula was used to apply the stem cell/collagen powder mixture into the furcation defects.

3.6. Stem cell isolation

The cells were isolated and the culture was modified after a protocol from Haddouti *et al*. (19). All tissue samples were cut down to small pieces under aseptic conditions in a laboratory. The tissue was incubated in DMEM-F12 (gibco, Carlsbad, USA) with an added 2.5. mg/ml of dispase (Sigma-Aldrich, St. Louis, USA) for 1 – 2 hours at 37 °C and 5 % CO2. After incubation the tubes were centrifugated at 400 x g for 4 minutes at 4 °C. The supernatant was discarded, the remaining tissues transferred into a cell culture flask (greiner-bio one GmbH, Kremsmünster, Austria) and 3 ml cell culture medium (DMEM-F12 including 10 % fetal bovine serum purchased from BIOCHROM, Berlin, Germany) and 1 % PenStrep (gibco, Carlsbad, USA) was added. The cell culture flask was incubated at 37 °C and 5 % CO2.

The following day, floating cells were removed and the medium was replaced with fresh medium. All

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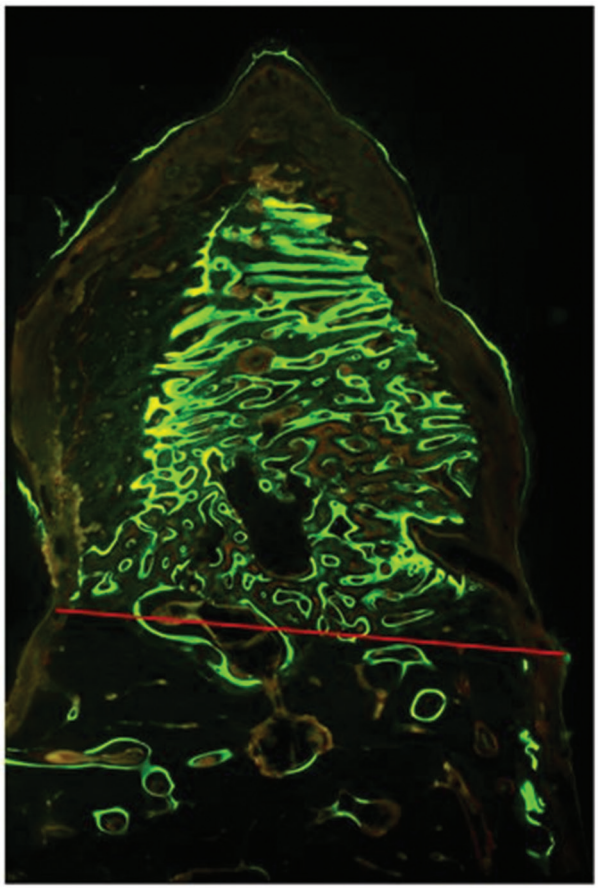

Figure 4. Fluorescence microscopy of furcation defect. A clear difference between newly formed bone (above red line) and older bone (below) with less bone formation is obvious as well as formation of calcified tissue formation on the root surface, magnification 20×, green = calcein green, red = alizarine complexone, orange = xylenol orange.

samples were checked for bacterial contamination. The flasks without bacterial contamination were incubated for 1 – 2 weeks and attached cells were fed with fresh medium every 3 days. The cells were cultured in DMEM-F12 and passaged with trypsin (gibco, Grand Island, NY, USA). For reimplantation 10^6 cells of passage 3 – 4 were applied. Stem cells were characterized flowcytometrically with the respective expressed surface molecules.

3.7. Retrieval of specimens and histological assessment

Upon premedication with ketamine (Pfizer AG, NY, USA) the animals were euthanized by an overdose of thiopental (Ospedalia AG, Hünenburg, Schweiz) 120 days after the last surgery. The oral tissues were fixed by perfusion with 10 % buffered formalin (Helm Austria GmbH, Wien, Austria) administered through the carotid arteries. The mandible was exarticulated and cut into segments, fixed in formafix 4 % for 7 days, kept in ethanol for 14 days and then block-embedded in technovit® (Heraeus, Hanau, Germany) 7200 VLC over 28 days. The specimens were cut into 250 µm-thick sections in the sagittal direction using a saw microtome (EXAKT Advanced Technologies GmbH Norderstedt, Germany) under permanent cooling. Finally, the specimens were dragged down to 15 µm and stained with toluidine blue.

The specimens were evaluated with respect to morphologic and morphometric aspects in an observer blinded fashion. A light optical microscope (Carl Zeiss, Axio Imager M2, Jena, Germany) with scanning stage was used and all samples were scanned at a magnification of 20 using a Axiocam MRC5 digital microscope camera. The evaluation and measurement of the samples occurred with the help of Axiovision (microscope software, Carl Zeiss Microscopy GmbH, Jena, Germany). The bottom of the defect was marked by the notches. The fluochrome labeling was used to determine the defect area, if the notches could not be identified in the histological samples. Newly formed tissues could be identified by fluorescent colors, which accumulated in tissues with a higher formation rate (Figure 4). The visualisation of the fluorescence labeling was observed via a fluorescence microscope (Carl Zeiss, 4×, Axiovert 40 CFL, Axiocam MRC5, Jena, Germany) with a filter of 490 – 520 nm especially excitating the calcein green fluorescence.

The total defect length was defined as the distance along the root, from one notch to the other (= 100 %). It was sectioned into different tissues such as new attachment (NA), connective tissue (CT), epithelial attachment (EA) and new cementum (NC) (see Figure 5A). Axio Vision SE64 Rel. 4.8. was used for the histomorphometric measurement. Each tissue was marked by different colours (Figure 5).

3.8. Statistics

The Kolmogorov-Smirnov test was used to prove normal distribution of the data. The ANOVA test followed to determine significant differences between the data of the measured defects' lengths. The level of statistical significance was set at $p \leq 0.0.5$. All data are presented as mean ± SD and graphs are crafted with GraphPad (GraphPad Prism 6 software, La Jolla, CA, USA).

4. RESULTS

All implanted materials increased the regenerative potential. Figure 6 shows the different filling materials and their newly formed new attachment. It is obvious that all experimental groups show an increased new attachment formation compared to the control group without any filling materials.

The collagen membrane plus GF achieved the highest new attachment level of approximately 77.3. % ± 20.3. % (Figure 7). The lowest new attachment level was

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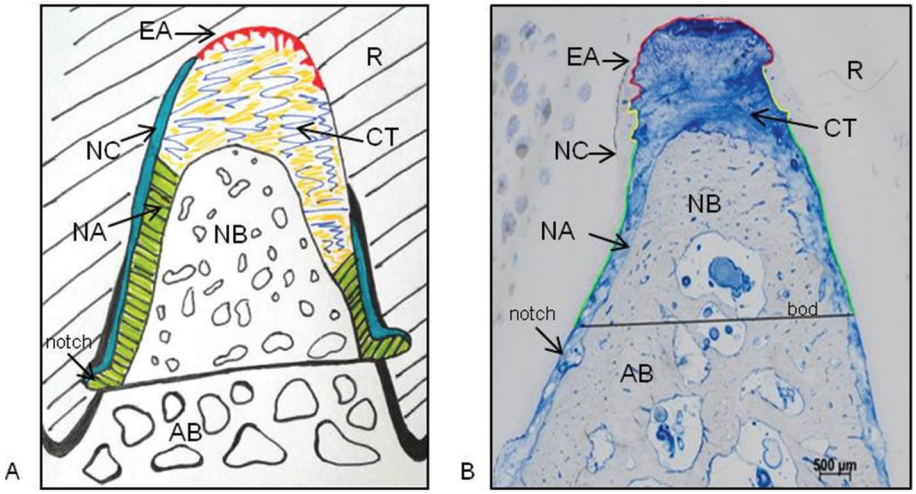

Figure 5. (A) Schematic and (B) histological drawing of length measurements. (A) Furcation defect with measured sections for histometric assessment; AB: alveolar bone, NB: newly formed bone, bod: bottom of defect, NC: newly formed cementum, NA: new attachment, EA: epithelial attachment, CT: connective tissue attachment, R: root. (B) Each color marks specific tissue: green: new attachment, red: epithelial attachment, yellow: connective tissue attachment.

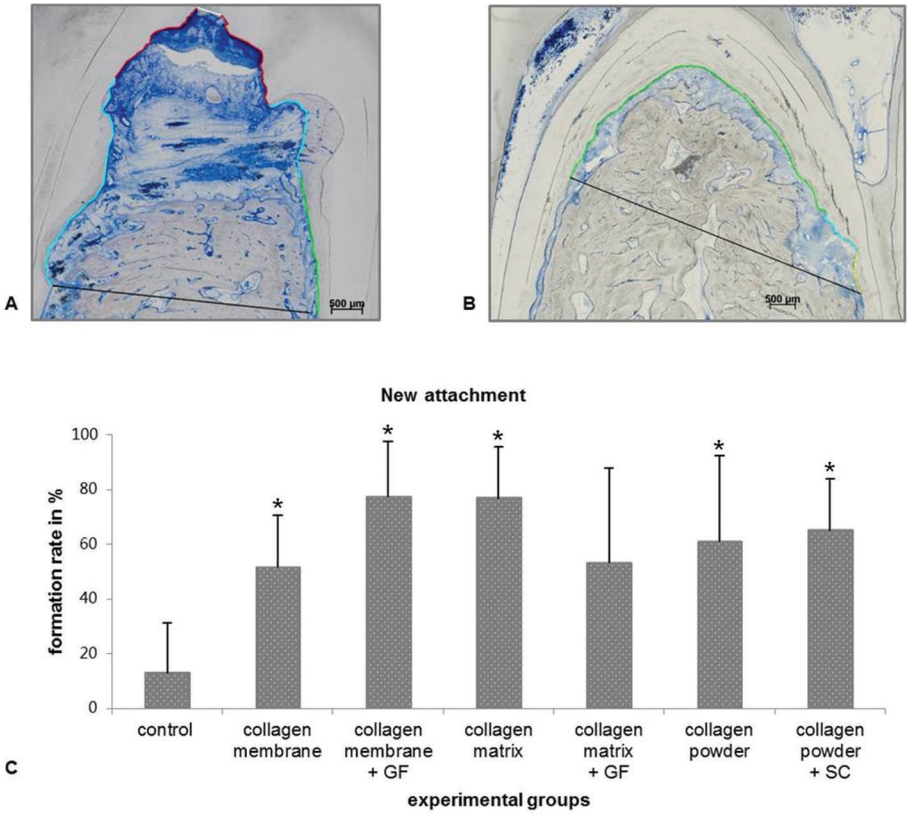

Figure 6. Formation of new attachment (NA). The new attachment is marked in green in histological measurements: (A) histological picture of control group: percentage of NA is about 13,0 %, (B) histological picture of collagen membrane plus GF: percentage of NA is about 77,3 %, (C) overview of NA of all implanted materials: y-axis shows percentages of measured tissue in relation to total defect length, x-axis shows different implanted materials and their significances in relation to the control group. P was set at $p \leq 0.0.5$ and all data are presented as mean ± SD.

found in the control group (13.0. % ± 18.0. %, Figure 8). In comparison to the control group, all tested implanted materials achieved higher new attachment levels. The second-highest result was achieved with the collagen

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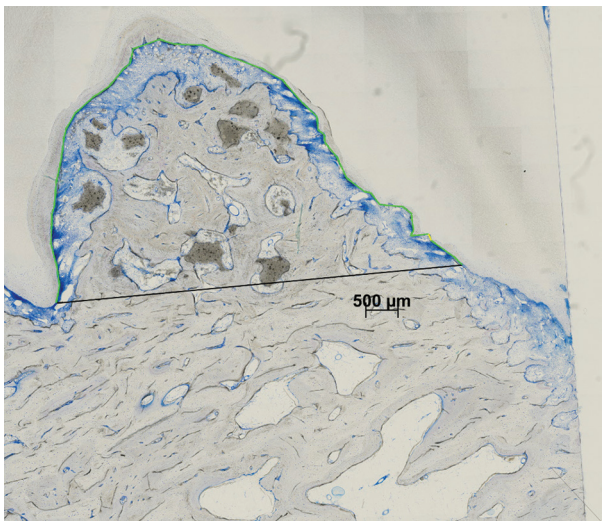

Figure 7. Histological picture of collagen membrane plus GF: NA is 77.3. % ± 20.3. %.

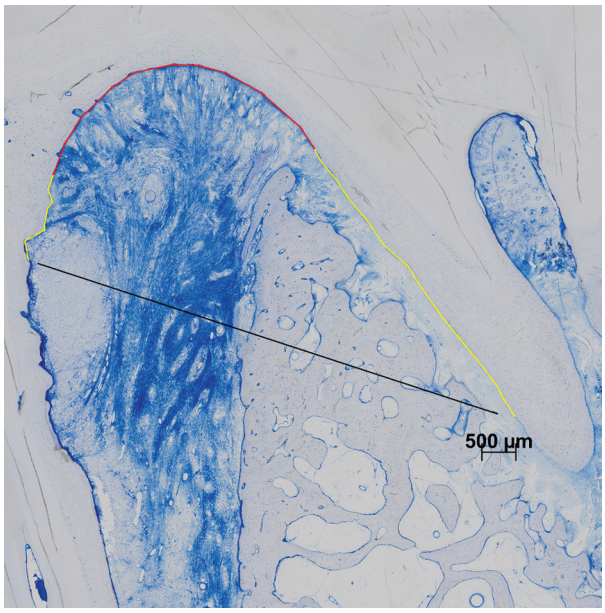

Figure 8. Histological picture of the control group: NA is 13.0. % ± 18.0. %.

matrix (76.9. % ± 18.6. %, Figure 9), followed by the collagen powder (61.1. % ± 31.4. %, Figure 10), the collagen powder plus PDLSCs (65.1. % ± 18.9. %, Figure 11) and the collagen membrane (51.7. % ± 18.8. %, Figure 12). All these results varied statistically significantly compared to the control, with the exception of the collagen matrix plus GF (53.3. % ± 34.6. %, Figure 13).

Furthermore, the epithelial attachment could be decreased in all experimental groups (Figure 14). In comparison to the control (epithelial attachment formation rate 39.5. % ± 28.6. %) four of the six experimental groups had a statistically significant decrease in epithelial attachment formation (collagen membrane: 4.7. % ± 9.3. %, collagen membrane plus GF: 0.0. % ± 0 %, collagen powder: 7.0. % ± 15.4. %, collagen powder plus SC: 2.2. % ± 4.9. %). Two groups showed a decreased epithelial attachment, but the results were not statistically significant (collagen matrix: 6.9. % ± 15.4. %, collagen matrix plus GF: 20.4. % ± 23.2. %).

4.1. Additional stem cells did improve periodontal regeneration, but not in a significant way

When assessing the carrier material solely by contrast with the carrier material plus stem cells, it was shown that the stem cells lead to an increased new attachment formation. Comparing the collagen powder alone or combined with the periodontal ligament stem cells, the new attachment level went from 61.1. % ± 31.4. % to 65.1 % ± 18.9. %, which means that there is a tendency to improve regeneration, but it is not statistically significant. However, the improvement of periodontal regeneration using periodontal ligament stem cells was not statistically significant in this study.

4.2. Additional growth factors did not significantly improve periodontal regeneration

Comparing the carrier materials for the growth factors solely with the carrier materials plus the additional application of the growth factors, no significant difference was found. The effect of the growth factors was controversial, because they had opposing effects. On the one hand, they improved regeneration (collagen membrane alone: 51.7. % ± 18.8. % versus collagen membrane plus GF: 77.3. % ± 20.3. %) while on the other hand the new attachment formation decreased (collagen matrix alone: 76.9. % ± 18.6. % versus collagen matrix plus GF: 53.3. % ± 34.6.%).

4.3. There was no significant difference between stem cells and growth factors

Considering the carrier materials by themselves and with the additional application of stem cells or growth factors, no significant difference in tissue formation was determined. All implanted materials improved regeneration, but the application of the stem cells or growth factors did not lead to a further significant enhancement in tissue formation.

4.4. Comparing the impact of the stem cells with that of the growth factors, there was no signifcant difference between them

Summarized, the addition of GF to the carrier materials resulted in an increased variability of the results with high standard deviations. The additional application of stem cells improved the outcome insignificantly. Both

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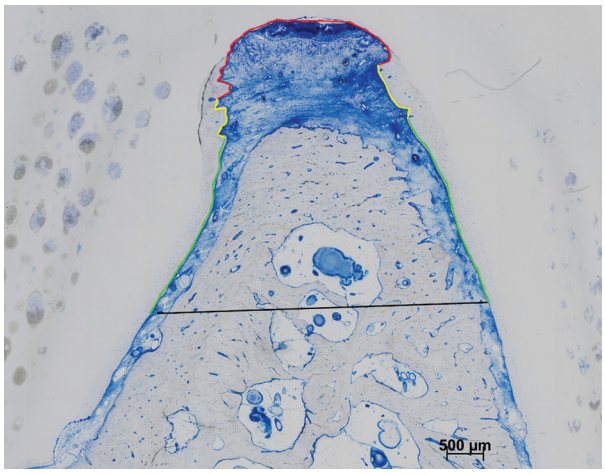

Figure 9. Histological picture of collagen matrix: NA is 76.9. % ± 18.6. %.

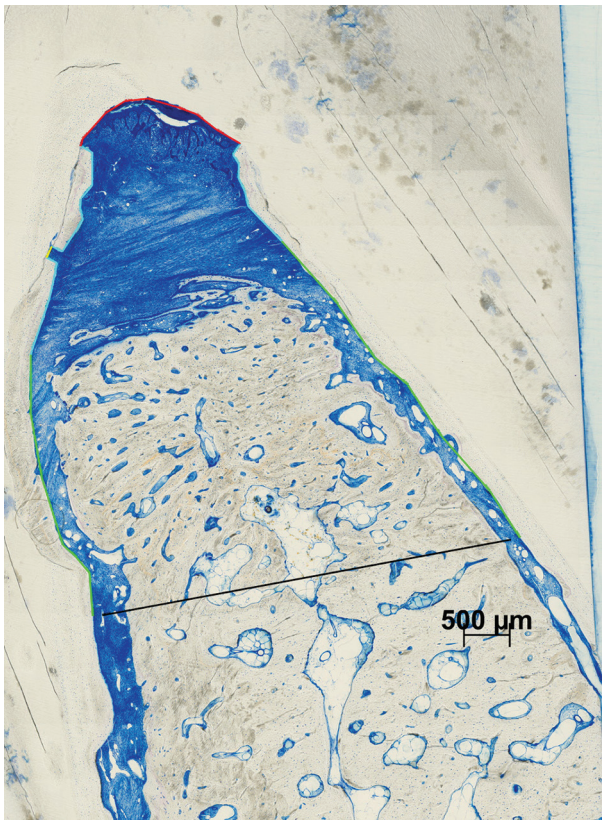

Figure 10. Histological picture of collagen powder: NA is 61.1. % ± 31.4. %.

materials tended to improve regeneration, but not in a statistically significant way.

5. DISCUSSION

In this study we used a minipig model to analyse the potential of different collagen carrier materials with or without periodontal ligament stem cells or a growth factor cocktail, to provide new treatment options for periodontal regeneration. To summarize, all carrier materials with or without stem cells or growth factors showed an increased formation of new attachment and a reduction of epithelial attachment. The supplementary application of stem cells or a growth factor cocktail additionally improved the healing processes, but not in a significant way. Collagen has previously been proven as a suitable carrier material for regeneration processes (20-22). On this account our study examined if the regenerative potential could be improved by the addition of growth factors or stem cells. Different kinds of collagens were used, a collagen matrix, a collagen membrane and a collagen powder. All materials could achieve a minimum 50 % higher new attachment formation compared to the control.

5.1. Carrier materials

There are different studies that confirm the regenerative potential of collagen. Kim *et al*. showed an enhanced bone/periodontal regeneration using an absorbable collagen sponge in beagle dogs (23). Kosen *et al*. filled class II furcation defects with collagen hydrogel/sponge scaffolds in beagle dogs and showed a significantly increased volume of reconstructed alveolar bone, cementum and new periodontal ligament (21). Another study showed similar results using a bovine hydroxyapatite/collagen block in one-wall intrabony periodontal defects in dogs (24). In two out of five groups a regeneration of the periodontal tissues could be achieved. It was shown, that collagen can be used for periodontal regeneration. Our study confirmed that collagen, implanted solely, increased new attachment formation. It has also been documented in several studies that the addition of stem cells and growth factors might further improve periodontal regeneration (22,25).

5.2. Stem cells

There are different kinds of dental stem cells, like dental pulp stem cells, stem cells from human exfoliated deciduous teeth or dental follicle stem cells (26). The stem cells used in this study were isolated from the periodontal ligament of extracted teeth.

The periodontal ligament stem cells were implanted using a collagen powder. The addition of the stem cells increased the extent of the new attachment. A reduction in the epithelial attachment formation rate was achieved as well. Our results confirm the outcomes of other studies. A systematic review from Bright *et al*. recapitulated that in 43 studies, using periodontal ligament stem cells in four species of animals (dog, rat, pig and sheep) and different sizes of surgical defects, 70,5 % of the results showed an statistically significant improved periodontal regeneration (27). Another study from Liu *et al*. from 2008 showed that periodontal regeneration could be significantly improved with the use of periodontal ligament stem cells in a porcine model (28).

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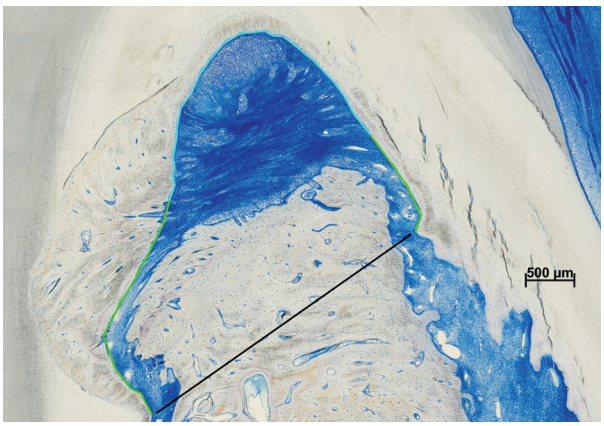

Figure 11. Histological picture of collagen powder plus PDLSCs: NA is 65.1. % ± 18.9. %.

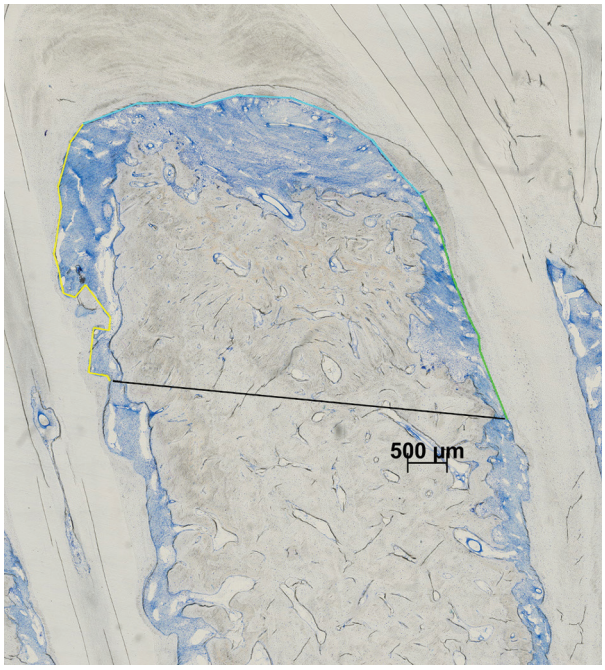

Figure 12. Histological picture of collagen membrane: NA is 51.7. % ± 18.8. %.

The findings from Liu *et al*. were proved by the study of Ding *et al*. from 2010, where allogeneic and autologous periodontal ligament stem cells were shown to have a significantly better periodontal tissue regeneration compared to control (29).

Dogan *et al*. and Akizuki *et al*. also cultured periodontal ligament stem cells *in vitro* for the treatment of periodontal defects *in vivo* (30,31). Both studies showed that PDL cells can prevent epithelial down growth and root resorption via the formation of connective tissue attachment which is characterized by parallel bundles resting on the root dentin. Another study by Tobita *et al*. demonstrated the effectiveness of adipose-derived stem cells. It was shown that eight weeks after the implantation of those stem cells a periodontal-like structure could be seen in rats (32). Our study showed a significant gain of new attachment by using PDLSCs when compared to the control, but there was no statistically significant difference when compared to the groups using GF or the carrier material alone.

5.3. Growth factors

The growth factor cocktail used in this study was implanted with different carrier materials and showed controversial results. The effect of growth factors is supposed to increase periodontal regeneration. Several studies showed that growth factors, based on their ability to regulate various functions of cells originating in the periodontal tissues, can improve periodontal regeneration. They may create an environment which is adjuvant to support a de novo tissue formation (33). It has been repeatedly shown that the use of growth factors improved regeneration. In various studies, the clinical attachment level improved three months after surgery while an increased rate of bone growth was also shown (34,35). In our study, the new attachment formation increased when combining the collagen membrane with the growth factor cocktail. On the other hand, the GF had an adverse effect when combined with the collagen matrix. In this experimental group the new attachment formation decreased. None of these effects was statistically significant. The growth factor cocktail that was used in this study had different impacts, depending on the carrier material used.

5.4. Methodological considerations

In this study, the stem cells and growth factors have been tested with different carrier materials. They were randomly put next to each other into furcation defects. The number of samples was limited by the regulatory guidelines of animal testings in Germany. Therefore there was a minimum of 4 defects per tested group.

The conclusion of this study is limited by several factors. The effectiveness of the stem cells and the growth factor cocktail is difficult to compare. Different collagen carrier materials were used for the growth factor cocktail and the SC. The growth factor cocktail was implanted either with the collagen matrix or the collagen membrane. However, the PDLCSs were only implanted with the collagen powder, which makes a direct comparison complicated. Earlier realized *in vitro* trials showed that the stem cells were not combinable with the collagen matrix. Reasons for this incompatibility might be that the pH-value of the collagen matrix was too low. Another reason might be the presence of endotoxins. Due to this incompatibility, the PDLSCs were only tested with the collagen powder. Pre-trials

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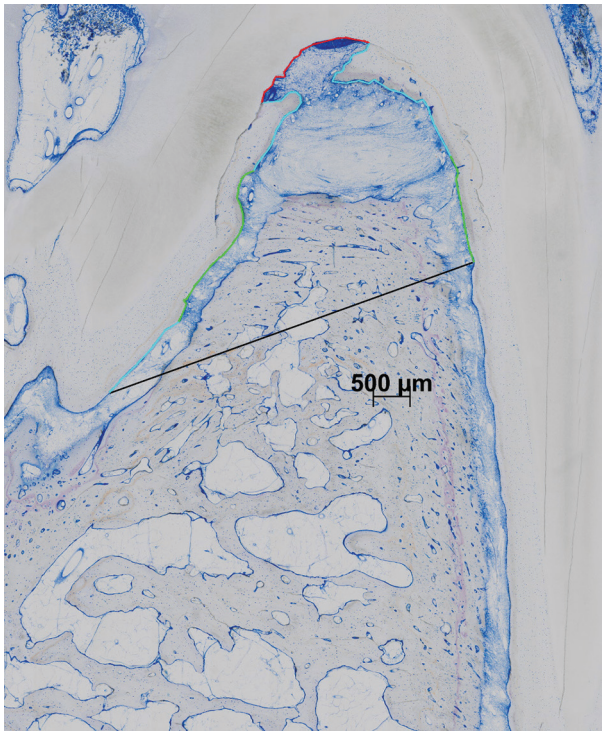

Figure 13. Histological picture of collagen matrix plus GF. NA is 53.3. % ± 34.6.%.

showed that the stem cells survived at least 24 hours when combined with the collagen powder. Therefore, the results showed an improved periodontal regeneration using the PDLSCs.

The growth factor cocktail used in this study may differ from growth factors used in other investigations and it does not provide any information about the effectiveness of the single growth factors used in this cocktail (16). Furthermore, the four different growth factors used in this cocktail, may potentially interact among themselves. Due to the fact that the growth factors were implanted with different carrier materials than the stem cells, it is challenging to compare their effectiveness. Nevertheless, since all implanted collagen materials lead to an increased new attachment formation (from 51 – 79 %), a tendency for the regenerative potential of the additional application of GF or SC can be drawn.

The animal experiments were performed on adult minipigs. Minipigs are omnivores and the anatomical structure of their teeth and periodontal apparatus resembles humans. The wound healing processes are also comparable (36).

6. SUMMARY

The use of stem cells alone to improve periodontal regeneration has been investigated in several studies with rats (37,38) while the efficacy of GF alone has been investigated in dogs (39) and humans (34). To the best of our knowledge there is no other study comparing the effectiveness of SC or GF in one study while using the same animal model. This study showed that the carrier materials alone or in combination with SC or GF can induce a significantly improved periodontal regeneration when compared to the non-treatment control. In contrast, the addition of SC to the carrier material did not improve the extent of the periodontal regeneration statistically significantly. The same was true for the GF. Furthermore, there was no significant difference between SC and GF.

7. CONCLUSION

In clinical practice, there are various factors affecting periodontal regeneration. Healing processes depend on the health condition, the metabolic processes, genetic factors and the immune system of the tested animal. Moreover, they can be influenced by the surgeon's abilities or his clinical experiences. Therefore, the regenerative processes *in vivo* cannot be compared with experimental studies with stem cells *in vitro.* Experimental investigations *in vitro* are subject to less environmental factors and individual variations, both of which have a high impact on *in vivo* research. Therefore, the positive effect of stem cells or growth factors shown *in vitro* may be overlayed by a significant variation of individual factors, which are also represented by the large standard deviations in this study. It was shown that with an increase in the complexity of a system, the variability increases.

Therefore, it might be reasonable to assume that the additional application of the growth factor cocktail or SC has a positive, but limited effect on regeneration *in vivo*, which is non-significant due to the clinical situation and the variety of factors influencing the wound healing. The PDLSCs, used in this study, were shown to improve periodontal regeneration, but relating to the complexity of this examination this improvement is not statistically significant.

The same effect was shown in other fields of medicine. In a study with rabbits, corneal transplants in combination with adipose tissue derived MSCs did not increase the survival of the transplants, but rather increased inflammation. This procedure led to a lower survival of the graft compared to sham-treated corneal transplants. The authors concluded that there are parameters like cell source, time of injection, immune suppression, number of cells etc which must be established before MSCs can be useful in corneal transplants (40). Another review questions the impact of bone marrow stem cells (BMSCs) used for kidney repair. Some studies have shown that BMSCs can differentiate into renal cells. Others doubt their efficacy (41). Stem cell research seems to be at a point where the basic approaches that could be achieved

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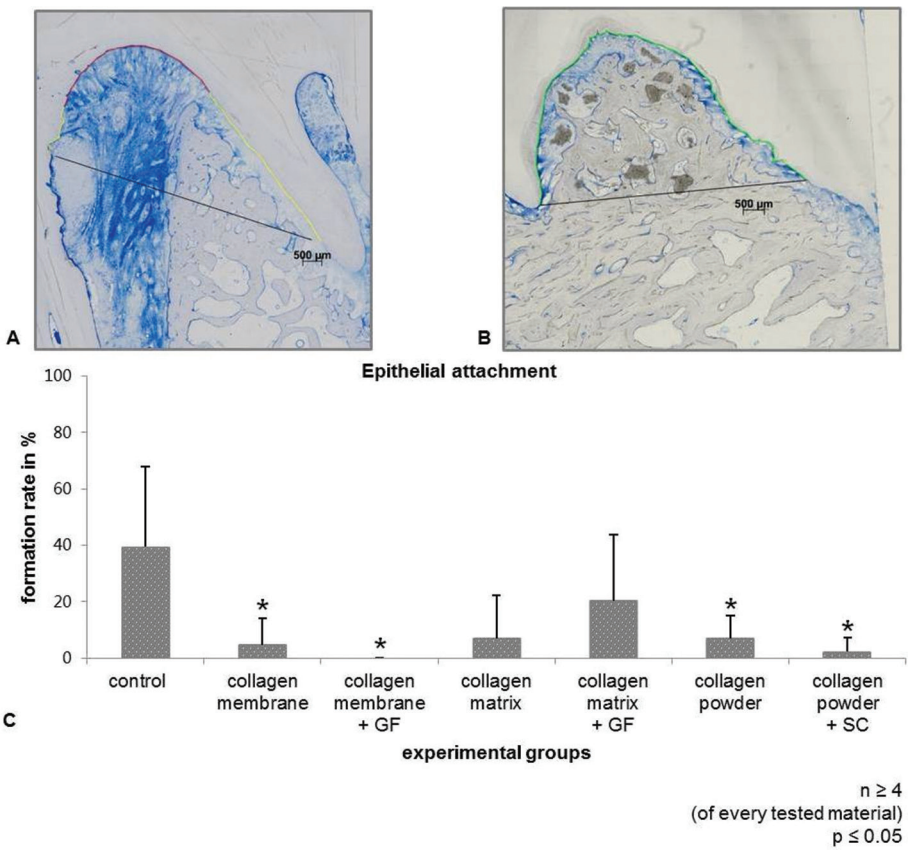

Figure 14. Formation of epithelial attachment (EA). The epithelial attachment is marked in red in histological measurements: (A) histological picture of control group: percentage of EA is about 40,0 %, (B) histological picture of membrane plus growth factors: percentage of EA is about 0 %, (C) overview of EA of all implanted materials: y-axis shows percentages of measured tissue in relation to total defect length, x-axis shows different implanted materials and their significances in relation to the control group. P was set at p ≤ 0.0.5 and all data are presented as mean ± SD.

over the last years need to be solidified for promising applications *in vivo* (42). Our findings indicate that results achieved *in vitro* or in testings with small animals – using SC and/or GF – cannot always be easily transferred into a clinical situation.

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Abbreviations: GF, growth factor cocktail; PDLSCs, periodontal ligament stem cells; SC, stem cells; GTR, guided tissue regeneration; PDL, periodontal ligament; DPSCs, dental pulp stem cells; PBS, phosphate buffered saline; NA, new attachment; EA, epithelial attachment; CT, connective tissue; NC, new cementum; BMSCs, bone marrow stem cells

Key Words: Periodontal Regeneration, Collagen, Stem Cells, Growth Factors, Minipigs

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