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Biomimetic Hybrid Scaffolds for Engineering Human Tooth-Ligament Interfaces

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Abstract

A major clinical challenge in the reconstruction of large oral and craniofacial defects is the neogenesis of osseous and ligamentous interfacial structures. Currently, oral regenerative medicine strategies are unpredictable for repair of tooth-supporting tissues destroyed as a consequence of trauma, chronic infection or surgical resection. Here, we demonstrate multi-scale computational design and fabrication of composite hybrid polymeric scaffolds for targeted cell transplantation of genetically modified human cells for the formation of human tooth dentin-ligament-bone complexes *in vivo*. The newly-formed tissues demonstrate the interfacial generation of parallel- and obliquely-oriented fibers that grow and traverse within the polycaprolactone (PCL)-poly(glycolic acid) (PGA) designed constructs forming tooth cementum-like tissue, ligament, and bone structures. This approach offers potential for the clinical implementation of customized periodontal scaffolds that may enable regeneration of multi-tissue interfaces required for oral, dental and craniofacial engineering applications.

Keywords

Tissue engineering; Rapid prototyping; Periodontal disease; Interfacial tissue formation; Regenerative medicine; Composite scaffolds

1. INTRODUCTION

Collectively, periodontal diseases afflict over 80% of adults worldwide and nearly 15% display severe disease concomitant with early tooth loss [1]. In periodontitis, the detrimental changes that the tooth-supporting tissues undergo are primarily the result of specific microbial challenges [2]. These challenges in a susceptible host disrupt the functional and structural integrity of the tooth supporting apparatus and may progress to affect a number of systemic

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conditions [3]. Therefore, the periodontium represents a critical barrier that if breached by invasive pathogens, triggering local and systemic inflammatory responses that characterize oral infection.

Structurally, regeneration of the lost periodontium involves the formation of new cementum, periodontal ligament (PDL) and alveolar bone. However, the proper interfacial connection of this multi-tissue complex is what determines its function and stability in health. Its strength and mechanical integrity is the result of adequate PDL-fiber orientation and its incorporation to the newly formed bone and cementum. This interconnection allows the periodontal system to dissipate and translate the mechanical stimuli that are generated from the tooth to the surrounding structures [4]. Biologically, this arrangement facilitates crucial cell-matrix interactions, which within a mechanically dynamic environment, determines normal dental-alveolar adaptive responses [5]. Current available regenerative therapeutic approaches show promising results [6–8]. However, complete regeneration and adequate fiber organization in large defects remains a challenging and unpredictable clinical dilemma [9].

In regenerative medicine, many different factors have been reported to promote multiple tissue integration and cell/tissue directionality [10–16]. Novel approaches, such as the use of multi-phasic scaffold designs as well as stem cell therapies represent a significant step forward in tissue engineering [13,14,17,18]. Today, the ability to establish a 3-dimensional polarity and patterning within a predetermined inherent scaffold geometry to guide and establish cell/tissue directionality is a feasible concept [15,19–21]. Cell-based research has started to focus on designing and developing various physical and geometric approaches using biomaterials [22, 23]. However, the orchestration of multiple tissue formation, spatial fibrous tissue organization, and endpoint functional restoration using a single *in vivo* scaffold system remains a significant challenge. To address these limitations, a computational topology design and a solid free-form fabrication technique was used to create a hybrid periodontal-inspired model system containing PDL-specific and bone-specific polymer compartments [24,25].

2. MATERIALS & METHODS

2.1. Hybrid scaffold design and fabrication

Periodontal ligament and bone architectures for the hybrid scaffold were designed and modeled with Unigraphics NX 5.0 (Siemens PLM software, Plano, TX USA). The designed structures were exported to the 3-D wax-printing system (ModelMaker II, Solidscape, Inc., Merrimack, NH USA) and manufactured using different wax molds (fig 1B). After dissolving the Protobuild (Solidscape, Inc.) of PDL mold by 70% ethanol, two different biopolymers poly (glycolic acid) (PGA; MW>100KDa, Polysciences Inc. Warrington, PA USA) and poly-ε-caprolactone (PCL; MW 43-50KDa, Polysciences Inc.). 25w/v% PGA was dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP, Sigma-Aldrich®, St. Louis, MO USA) solvent and the solution was cast for PDL interface architecture. 25 w/v% PCL solution in acetone (Sigma-Aldrich®) was cast in the bone architecture mold. These 2 different manufactured and fabricated architectures were assembled with PCL thin film membrane and BioAct® VSO (Petroferm Inc. Gurnee, IL USA) was used to remove Protosupport (Solidscape Inc.) for 2 days. The rest of Protosupport and BioAct VSO were dissolved in 100% ethanol overnight and hybrid scaffolds were stored in 70% ethanol.

2.2. Human tooth dentin slice preparation

Healthy human teeth were extracted from patients as previously described by the University of Michigan-Institutional Review Board (UM-IRB)-approved protocol. Approximately $3.0 \times 4.0 \times 0.8 \text{ mm}^3$ dimensioned dentin blocks, which were fit to PDL interface of the hybrid

scaffold, were sliced and surface-treated by 37% orthophosphoric acid to expose dentinal tubule topology and promote fibrous tissue attachment.

2.3. Cell cultures and gene delivery

Primary human gingival fibroblast (hGF) cells were provided as a kind gift from professor Martha Somerman (University of Washington, Seattle, WA USA). Passages 4–6 hGF cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco BRL Life Technologies Inc., Grand Island, NY USA) supplemented with 10% fetal bovine serum (FBS; Gemini Bio-Products, Woodland, CA USA), antibiotics (100 units/ml penicillin and 100 µg/ml streptomycin) and 2mM glutamine. During culturing in a humidified atmosphere of 5% CO₂ in air at 37°C, the hGF cells were transduced with AdCMV-BMP-7, recombinant adenovirus-encoding murine bone morphogenetic protein-7 (BMP-7), at a multiplicity of infection (MOI) of 500 and incubated for 1 day before cell seeding into the bone portion of hybrid scaffolds. Passages 4–6 human periodontal ligament cells (hPDL) were derived from tooth biopsy samples from 3 healthy human patients and cultured in media.

2.4. Cell seeding in the hybrid scaffolds

Bovine plasma fibrinogen (Sigma-Aldrich®) was dissolved in DMEM to make 5 mg/ml concentration and sterilized with a 0.2 µm syringe filter (Nalgene®, Rochester, N.Y. U.S.A.). Bovine plasma thrombin (Sigma-Aldrich®) was dissolved in Hanks' Balanced Salt Solution (HBSS, Invitrogen™) at 100 U/ml concentration. For the bone region, 2.4×10^5 BMP-7-hGF modified cells in the bone region and 1.4×10^5 hPDL cells in the PDL interface of hybrid scaffolds were suspended within fibrinogen solution. 1.6 µl thrombin was pipetted on the PDL interface and 8.0 µl hPDL-fibrinogen solution was dropped. After the gelation of the PDL interface, the treated tooth dentin slice was immediately positioned on the PDL scaffold. For BMP-7-hGF cell seeding, 3.0 µl thrombin solution was pipetted inside of the bone architecture and 15.0 µl hGF + fibrinogen solution was drop-wise added for fibrin gelation.

2.5. In vivo experimental design

Two different surgical pockets on the dorsa of immunodeficient 6 week-old NIH III nude mice (approximately 50–55g from Charles River Laboratories International Inc., Wilmington MA USA) were created. Four different groups were designed with 3 and 6 week time-points in order to implant surgically created subcutaneous pockets of the nude mouse with sample n-values=8–9/group for each time point. The experimental groups consisted of 1) no cell seeding, 2) hPDL cell-seeded in PDL interface, 3) Ad-BMP-7-hGF cell seeded in bone region, and 4) hPDL and Ad-BMP-7-hGF cell seeded in PDL interface and bone region of hybrid scaffolds, respectively. Ad-BMP-7-hGF was the adenovirus encoding BMP-7 transduced human gingival fibroblast cells. Under isofluorane anesthesia, dentin-associated hybrid scaffold complex was implanted subcutaneously into a surgically-created pocket and the incisions were approximated using surgical staples. All animals were euthanized with carbon dioxide (CO₂) prior to specimen harvest. All animal surgeries were performed under a protocol approved by the University of Michigan-University Committee on Use and Care of Animals (UM-UCUCA).

2.6. Quantitative micro-computed tomography (Micro-CT)

The harvested specimens were fixed in 10% buffered formalin phosphate solution for 1–2 days and transferred in 70% ethanol. The tissue-fixed specimens were scanned by micro-CT (GE Healthcare Inc., London, ON Canada) with $18 \times 18 \times 18 \mu\text{m}^3$ voxel size. Based on the Hounsfield unit (HU) scale, mineralized tissue regeneration was quantified around the hybrid scaffold, bone region and PDL interface below the treated human tooth dentin slice by MicroView Analysis+ 2.1.2 (GE Healthcare Inc.). The region of interests (ROIs) for PDL

interface and bone region were $3.5 \times 4.5 \times 0.8\text{mm}^3$ and $5.0 \times 6.0 \times 3.0\text{mm}^3$, respectively to assess quantification of mineralized tissue formation.

2.7. Histomorphometry for cementum-like tissue and tissue orientation formation

After the micro-CT scanning, harvested specimens were decalcified with 10% EDTA (Ethylenediaminetetraacetic acid, Sigma-Aldrich®) for 2 weeks and then paraffin-embedded for histology sections for hematoxylin and eosin (H&E) staining. Image-Pro plus software (Media Cybernetics Inc., Bethesda MD USA) was utilized to calculate length percentage of cementum-like layer on the human tooth dentin slice. The % length of cementum-like tissue formation (l_{cementum}) was determined by comparing the newly-deposited cementum length and the total dentin surface (l_{dentin}), which contacted to PDL interface of the hybrid scaffold.

$$\% \text{ of } l_{\text{cementum}} = \left[\frac{l_{\text{cementum}}}{l_{\text{dentin}}} \right] \times 100$$

The orientation of fibrous cells and connective tissues in PDL interface was analyzed with four different indices, percentile of cellularity and orientation score (Fig. 4b). The best orientation was the perpendicular alignment to the dentin surface.

2.8. Scanning electron microscopy (SEM)

The scaffold constructs and dentin slices were washed, sonicated, dehydrated and the surface prepared for evaluation using the Environmental Scanning Electron Microscope (Philips XL30E SEM FEI Company, Hillsboro, OR USA). Briefly, after dehydration, the specimens were attached to a stub and sputtered with gold/palladium. The gold/palladium-coated specimens were examined by use of a FEI/Philips XL30 field emission environmental scanning electron microscope (SEM).

2.9. Immunofluorescence staining

Tubulin and 4',6-diamidino-2-phenylindole (DAPI) staining were performed. Briefly, the samples were dissected and fixed in 4% paraformaldehyde at 4°C overnight, demineralized in 10% EDTA solution over 3 weeks, dehydrated, embedded in paraffin and processed for sectioning (6µm thickness). Fluorescence staining to tubulin (1:100 dilution; Abcam, Inc. Cambridge, MA) was performed on paraffin sections using an affinity purified rat monoclonal antibody. Immunological reaction was visualized by using a rabbit polyclonal secondary antibody to rat conjugated to Fluorescein isothiocyanate (FITC) (1:200 dilution; Abcam, Inc. Cambridge, MA). The Sections were then treated with an antifade agent containing DAPI (ProLong Gold antifade reagent with DAPI; Invitrogen Corporation; Eugene, OR) and covered with glass coverslip. The stained slides were imaged using an OLYMPUS Fluroview 500 confocal microscope (Olympus America Inc; Center Valley, PA).

2.10. Customized, reversed-engineered scaffold design

After iCAT-CT (Xoran Technologies® Inc.; Ann Arbor MI USA) scanning of a porcine periodontal defect site with the 200µm voxel-size resolution, the DICOM file was transferred to a STL file format to import CAD-based Unigraphics NX 5.0. Based on the tooth root-surface, micro-channels perpendicularly oriented to the root surface were designed and booleaned with STL-formatted in order to generate a similar surface-morphology. Approximately, the dimension of PDL interface was 300µm-thick (Fig. 5; red-colored architecture) to cover from the apical side of periodontal defect to cemento-enamel junction (CEJ). After the hybrid scaffold, rapid prototyping technology was utilized to manufacture the molds to cast polymer.

25wt/v% PCL solution was casted into to printed wax molds and fabricated polymeric scaffold was placed to the periodontal defect to scan micro-CT to evaluate adaptation of defect geometry.

2.11. Adaptation evaluation of Hybrid scaffold-tooth root surface using the contrast agent and micro-CT

After the customized hybrid scaffold was designed and manufactured, 35% barium sulfate (BaSO_4) in distilled water was used to coat the 25% PCL hybrid scaffold to obtain the higher intensity and grayscale Hounsfield Unit. Surgically created periodontal defect region was harvested and scanned with/without the scaffold using micro-CT, which set up for $25 \times 25 \times 25 \mu\text{m}^3$ -voxel size resolution. On the 2-D coronal cross-sectioned view, 3.00mm distance from the interface between the dental pulp and tooth dentin was selected to generate the grayscale-based histogram. This region covered from the tooth root, PDL interface, and middle of bone region. Adaptation ratio was linearly calculated using the entire length of PDL interface (d^{total}) and the gap distance (d^{gap}) between tooth dentin surface and the surface of PDL interface architecture in the hybrid scaffold.

$$\text{Adaptation Ratio} = \left[\frac{d^{\text{total}} - d^{\text{gap}}}{d^{\text{total}}} \right]$$

The scaffold had six different layers and each layer had three PDL channel-like structures (n=3 per layer).

2.12. Statistical analysis

The PASW Statistics 17.0 (SPSS Inc. Chicago, IL USA) was used for statistical analysis. Nonparametric two-tailed Kruskal-Wallis one-way ANOVA for unequal variance (Supplementary Table S1). After the overall determination of statistical results from the Kruskal-Wallis test, pair-wise Mann-Whitney U-test were utilized to define the statistical differences among groups. We demonstrated the results were significantly different with the α value set at 0.05 level of significance.

3. RESULTS

A 0.8mm thickness PDL interfacial structure was designed with multiple perpendicularly oriented channels, devised for the guidance of fibrous connective tissue formation and alignment of fibroblast-like cells (Fig. 1a). The structure was fabricated in 25% poly(glycolic acid) (PGA), a hydrophilic and rapid degradable biomaterial. For the bone compartment, a global porous geometry with approximately $0.75 \times 0.50 \times 0.05 \text{ mm}^3$ dimensions using 25% poly- ϵ -caprolactone (PCL) was designed (Fig. 1a). The PDL and bone components were then fused with a 15% PCL thin layer to form one single hybrid scaffold structure.

The new scaffold design was evaluated using an *in vivo* model system. For this model, acid-treated human tooth dentin slices were sectioned to fit the PDL dimension and provide an avascular tooth surface with close proximity to the PDL microchannel-grooved scaffold design (Fig. 1b). The four different hybrid scaffold-dentin complexes were subcutaneously implanted in the dorsa of immunodeficient mice. The bone and PDL regeneration capacity was evaluated and the feasibility to adapt the model hybrid scaffold designed to the irregular periodontal defect topography was also determined.

Micro-computed tomography (micro-CT) and serial histological sections were utilized for the qualitative and quantitative assessment of the mineralized tissue formed and to evaluate potential invasion of bone within the PDL region (Fig. 2; [26]). The 3-D reconstructed images provided qualitative information regarding the patterning and spatial distribution of the newly formed bone in relation to the scaffold geometry (Fig. 2; colorized micro-CT images). Bone volume fraction (BVf) and bone mineral density (BMD) were quantified using grayscale Hounsfield Units (HU). Specified regions of interest (ROIs) for PDL interface and bone region were created in $3.5 \times 4.5 \times 0.8\text{mm}^3$ and $5.0 \times 6.0 \times 3.0\text{mm}^3$ dimensions, respectively. A robust mineralized tissue formation was observed in the groups containing the transduced osteogenic factor. These groups within the bone region demonstrated statistically significant higher values for BVf and BMD (* $p < 0.05$ and ** $p\text{-value} < 0.01$; Fig. 3a and b). On the other hand, the only bone formed outside the bone region, was localized to the peripheral surface of the scaffold and dentin construct. No bone was found to be invading the PDL at either of the two time points for non BMP-treated constructs (Supplementary table S1). Histological evaluation of the PDL and bone regions provided further evidence of the lack of osteogenesis within the PDL region while corroborating the presence of bone within the other compartment (Fig. 2).

Hematoxylin and eosin (H&E) serial images of the PDL-dentin interface were histomorphometrically evaluated to determine the length of new cementum formation (Fig. 3). At 3 weeks, limited evidence of cementum-like tissue deposition was observed. However, at 6 weeks the length of cellular cementum in the groups transduced by with BMP-7 were significantly greater compared to the other groups. (Fig. 3c and d, * $p = 0.024$ and ** $p = 0.007$). The histomorphometric analysis showed that new cementum-like tissue formed in 20.62% of the hPDL/BMP-7-hGF cell-seeded group and 17.70% of BMP-7-hGF cell-seeded group, compared with 0% and 1.2% of the no cell and hPDL-seeded groups, respectively (Fig. 3c). Interestingly, the newly formed cementum was observed in association with vascular structures and fibrous connective tissue (Fig. 3d). The associated fibrous tissue is speculated to represent a transition cue of the cementogenesis process on the dentin surface and the initial integration of fibrous bundles to the dentin slice [27].

These findings are particularly important as they emphasize the potential biological effect of the hybrid scaffold system. The designed PDL interface architecture was initially conceived to increase angiogenic development and enhance biological molecule diffusivity. This theoretical concept is supported by the *in vivo* findings, which reflect an increased number of vascular structures and mature fibers oriented along the patterned surface scaffold architecture (Figs. 2 & 4). This was especially observed in the two different hPDL cell-seeded groups. At 3 weeks, these observations were primarily reflected in the BMP-7 transduced groups (Fig. 4). However, by the 6th week, a more generalized increased level of tissue orientation was observed in different proportions for all groups (Fig. 4).

To evaluate the viability of the human-seeded cells within the scaffold complex, the harvested constructs were immunostained for human leukocyte antigen-A (HLA-A). Distinct membrane staining was noted in the PDL and bone regions of the groups where human cells were transplanted. These groups of cells within the PDL were preferentially localized along the polymer architecture and at the center of more mature fibrillar structures. Within the bone scaffold region, the HLA-A positive cells were clearly localized primarily around the new bone surface and presented an osteoblast-like phenotype. Multiple osteocyte like cells were also positive for HLA-A (Supplementary Fig. S1).

4. DISCUSSION

The current system, however, still has limitations compared to the highly complex native multi-tissue formation [12–14], especially bone-PDL-tooth complex [9,28,29]. One of the most

important variables is the lack of a mechanically-modulated environment as well as a symmetric design which does not completely reflect the irregular and complex morphology of typical periodontal defects [4]. Therefore, supported by the biological advantages demonstrated in this novel hybrid scaffold, a more periodontally relevant design was constructed using surgically created defects in porcine mandibulae to determine the ability to conform to the anatomical specifications of the periodontium (Fig. 5). The customized scaffold displayed perpendicularly oriented internal channel-structures within the PDL portion and a similar bone topology in the open-box format to follow and coalesce with the adjacent residual bone. The inter-root defect scaffold was designed to possess two separate components with a key (buccal)–lock(lingual) system to make easier the assembling and adaptation through the defect region (Fig. 5a). The adaptability/fitting of the fabricated scaffold was evaluated using 3-D and cross-sectional analysis to establish the approximation of the scaffold construct in relation to the periodontal tissues and defects (Fig. 5b–d). This possibility of customization and, therefore, guidance of each tissue represents an additional advantage with a significant clinical relevance.

Together, these observations support the added value of designing a compartmentalized hybrid scaffold with biomimetic architecture to influence cell behavior and tissue orientation. The combined hPDL cells and Ad-BMP-7-hGF cells not only surpassed most of the measured variables when compared to the other test groups, but also produced a more predictable formation of tissue similar to healthy periodontium. Therefore, our principal finding was that a combinational system of hPDL fibroblast-like cells and osteogenic stimulation can enhance the regeneration of the multi-layered periodontal complex. This approach offers strong potential for an “off-the-shelf scaffold construct for repair of oral and craniofacial defects.

5. CONCLUSIONS

We demonstrate the consistent generation of newly-formed tissues possessing interfacial neogenesis of parallel- and obliquely-oriented ligamentous fibers that sprout and traverse through the polymer designed constructs forming tooth cementum-like tissue, ligament, and bone structures. This approach offers potential for the clinical implementation of customized periodontal scaffolds that may enable regeneration of multi-tissue interfaces required for oral, craniofacial, and periodontal engineering applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

a) Schematic illustration of the 3-D wax printing system and dimension of hybrid scaffold shows polymeric architecture manufacturing. For the PDL interface, column-like structures were 0.8mm diameter and 0.3mm exposed heights and casted using PGA-HFIP solution. For the bone region of the hybrid scaffold, PCL-acetone solution was used for casting. PCL-acetone, pasted on the PCL-casted mold and PDL interface architectures were placed on it.

b) After the acid-treatment of human tooth dentin slices, the complex with a polymer-casted hybrid scaffold and a dentin slice was assembled using fibrin gel with or without cells. The left is the 3-D designed hybrid scaffold and the right panel is the micro-CT scanned and 3-D reconstructed hybrid scaffold and a dentin slice. The scale bar: 50μm.

Figure 2. 3-D reconstructed colored micro-CT images and hematoxylin and eosin (H&E) stained histology

The mineralized tissue (blue-colored) was formed around the hybrid scaffolds and there was no ankylosis, bone fusion to the dentin surface (white-colored). Red and blue dashed lined-boxes represent the PDL interface and bone regions, respectively. H&E stained histology slices showed PDL interfaces and bone region tissues to evaluate fibrous tissue orientation along the column-like structures in PDL interface, which were designed with perpendicular direction to the dentin surface. At 6 weeks, hPDL cell seeded specimens demonstrated perpendicular orientation to the dentin slices and along the column-like structures with limited evidence of cementum-like cells on the dentin surface. Yellow dash-line is the borderline of channel-type

PDL architecture and black arrowed lines represent the fibrous cell/tissue directionality following the wall of PDL interface structure. Red triangles indicate the blood vessels and yellow triangles point cementum-like tissue layer or cell deposition for cementogenesis on the dentin surface. The scale bar: 50 μ m.

Figure 3. Quantitative analysis of micro-CT and histomorphometry for cementum-like tissue length

At 3 week time point, **a**) bone volume fraction (BVF) and **b**) bone mineral density (BMD) at 3 and 6 weeks were measured and analyzed statistically for bone regions of the hybrid scaffolds. **a and b**) In the aspects of BVF and BMD, there were statistically significant differences between the osteogenic factor and non-osteogenic factor groups in bone regions at 3 weeks (** $p < 0.01$) and 6 weeks (* $p < 0.05$ and ** $p < 0.001$). **c**) The cementum-like tissue length percentage represented of mineralized layer deposition on the dentin surface at 3 and 6 weeks. Full surface length of dentin slice, which faced to the PDL interface was measured and divided the measured the cementum-like tissue length (%). At 6 weeks, hPDL/BMP-7-hGF cell seeded group had statistically significant differences with no cell and hPDL cell seeded groups (* $P = 0.02433$ and ** $p = 0.00722$). **d**) Qualitative results using H&E staining for the cementum-like tissue formation on the dentin surface. In 3 weeks, there was limited evidence of the mineralized layer formation but, in 6 weeks, significantly increased mineral deposition can be determined with the cementocyte-like cell embedded indicated by yellow triangles. For all statistical data analysis, two-tailed Kruskal-Wallis one-way ANOVA test and Mann-Whitney U-Test were utilized and data were mean $\pm$ standard error of mean (S.E.M.). scale bar: 50µm.

Figure 4. Cellularity and cell/tissue orientation in PDL interface using H&E staining and Immunofluorescence images

The fibrous tissue orientation was measured by semi-quantitative analysis with four different indexes; no cellularity, cellularity without fiber formation, cellularity with disorganized fiber, and cellularity with perpendicularly oriented fiber formation to the dentin surface. The H&E staining pictures are the representatives for these four indices. The percent number of the semi-quantitative analysis was calculated with total number of samples in each group/time point and the number of each observation index. Immunofluorescent images represent four typical examples of indexes with DAPI (blue) and tubulin (green) staining in cytoplasm. Scale bar: 50 μ m.

Figure 5. The reverse-engineered periodontal defect-fit scaffold modeling and the adaptation of customized designed scaffold on the root surface

a) The computer-aided design (CAD)-based software, NX5 was utilized to create PDL (red-colored) and bone (blue-colored) interfaces of the hybrid scaffold. The anatomical defect-fit scaffold had the perpendicular oriented PDL internal channel-structures and topological similarities of the periodontal defects. The Furcation defect design had separated two different parts with key (buccal)–lock (lingual) system to make easier assembling and implanting through the buccal-lingual penetration defect region. **b–c)** The red line was porcine mandible image with the customized scaffold and the blue line was the exposed periodontal defect site. **b)** The histogram represented the 99.9% adaptable scaffold to the root surface. The measured length was 3.00mm and scaffold was coated by 35% BaSO₄ solution. The yellow lines on the 2-dimensionally digitized slices represented the measured regions with 3.00mm length from the dentin (dental pulp side) to the middle of defect site. The abbreviations were that AB: alveolar bone, R: tooth root, and Sc: hybrid scaffold. **c)** The histogram was from 83.5% adaptable scaffold image. The concaved region of the red line can represent the gap distance

(d^{gap}) between tooth root surface and PDL interface scaffold. **d)** Based on the method in Figure 5-c and d, total PDL interface length (d^{total}) and d^{gap} were linearly measured and the adaptation ratio was calculated in each layer, which had 3 different channel-type structures. There was no statistically significant difference (N.S.) among 6 different layers ($p=0.1143$) and the range of adaptation was $83.3\% < \text{mean value of adaptation ratio} < 99.0\%$ and data were mean $\pm$ standard deviation (S.D.). For the statistical analysis, the nonparametric Kruskal-Wallis one-way ANOVA test was used.