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Peptide Coatings Enhance Keratinocyte Attachment towards Improving Peri-implant Mucosal Seal

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Abstract

There is critical need for preventing peri-implantitis as its prevalence has increased and dental implants lack features to prevent it. Research strategies to prevent peri-implantitis have focused on modifying dental implants to incorporate different antimicrobial agents. An alternative strategy consists of barring the expansion of the biofilm subgingivally by forming a long-lasting permucosal seal between the soft tissue and the implant surface. Here, we innovatively biofunctionalized titanium with bioinspired peptide coatings to strengthen biological interactions between epithelial cells and titanium surface. We selected laminin 332- and ameloblastin-derived peptides (Lam, Ambn). Laminin 332 participates in the formation of hemidesmosomes by keratinocytes and promotes epithelial attachment around teeth; and ameloblastin, an enamel derived protein, is involved in tissue regeneration events following disruption of the periodontium.

Lam, Ambn or combinations of both peptides were covalently immobilized on titanium discs. Successful immobilization of the peptides was confirmed by contact angle goniometry, X-ray photoelectron spectroscopy and fluorescent labelling of the peptides. Additionally, we confirmed the mechanical and thermochemical stability of the peptides on Ti substrates. Proliferation and hemidesmosome formation of human keratinocytes (OKF6/TERT-2) was assessed by immunofluorescence labelling.

The peptide-coated surfaces increased cell proliferation up to 48h in culture compared to control surfaces. Most importantly, formation of hemidesmosomes by keratinocytes was significantly

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increased on surfaces coated with Ambn+Lam peptides compared to control ($p<0.01$) and mono-peptide coatings ($p<0.005$). Together, these results support the AMBN+LAM multi-peptide coating as a promising candidate for inducing perimucosal seal around dental implants.

Keywords

Keratinocytes; Peri-implantitis; Epithelia; Peptides; Ameloblastin; Laminin; Hemidesmosome; Dental Implants

1. INTRODUCTION

Peri-implantitis is an inflammatory process involving the soft tissues and supporting bone. The primary etiology of peri-implantitis is bacteria that colonize the exposed surface of the dental implant forming a biofilm.¹ The prevalence for peri-implantitis has been reported as ranging from 11 to 47%.² Consequently, there is critical need for development of strategies to prevent peri-implantitis and its' long-term consequences. The maintenance of a long-lasting physical and structural barrier between the soft tissue and implant collar or abutment surface has recently been proposed as a way to prevent peri-implantitis.³ This preventative strategy relies on strengthening the interaction between epithelial cells and the titanium or zirconia surface to impede the expansion of the biofilm subgingivally.

In nature, the integrity of the tooth-soft tissue interface is maintained via a specialized epithelial structure, referred to as junctional epithelium (JE). The JE is structurally similar around implants and teeth and attaches to both in a similar way.⁴ At the JE the deposition of the basement membrane on the enamel or implant abutment surface allows the establishment of hemidesmosomes (HDs).⁵ HDs are highly specialized integrin-mediated epithelial attachment structures responsible for the attachment of epithelial cells to the basement membrane and the tooth or implant abutment surface.^{5, 6} However, in contrast to what occurs in teeth, HDs have been only observed in the apical portion of the junctional epithelium around implants, which compromises the ability of the coronal portion of JE to serve as an adequate seal against the biofilm growth and expansion.

This reduced presence of HDs around implants is linked to a reduced expression of laminin 332 in the peri-implant JE.⁷ Laminin 332 is expressed and deposited by migrating oral and epidermal keratinocytes⁸ and has a key structural role in the formation of hemidesmosomes via $\alpha 6\beta 4$ and focal contacts via $\alpha 3\beta 1$ integrins at the keratinocytes' membrane.^{9, 10, 11} Previous studies have focused on improving soft tissue/implant biological interactions by coating titanium surfaces with laminin 332 or other proteins participating in the cascade of events during formation of the peri-implant/tooth seal.³ Early efforts with physisorbed laminin 332 coatings demonstrated beneficial effects on in vitro keratinocyte responses.^{12, 13} However, this approach led to unpredictable density and retention of the biomolecules on the implant surface with rapid loss of their bioactive properties. Furthermore, the use of laminin from animal sources raises immunological concerns and might result in fast protein degradation. By contrast, immobilization of biomolecules on titanium substrates via covalent bonding results in more robust and stable anchoring of the molecules.¹⁴

Bioactive oligopeptides can be used to overcome the aforementioned limitations.³ The 14-amino-acid peptide, PPFLMLLKGSTRFC (Lam) has been isolated from laminin 332 and has an active site for integrin $\alpha 3 \beta 1$ binding.¹⁵ Conjugating this peptide to a polyelectrolyte multilayered coating on titanium improved *in vitro* keratinocyte attachment, but did not have a significant *in vivo* effect on soft tissue attachment.¹⁶ Ameloblastin is a member of the family of enamel matrix proteins and involved in the secretory stage of ameloblasts during tooth formation.^{17, 18} Recently, Nishio et al. reported that Hertwig's epithelial root sheath cells expressed ameloblastin five days after disruption of the periodontium¹⁹ and Hasegawa et al. found that epithelial cell rests of Malassez, which have been suggested to play a pivotal role in periodontal regeneration²⁰, also expressed ameloblastin during the stage of cementum repair.²¹ Wazen et al. reported dental and junctional epithelium defects, characterized by an interdental junctional epithelium with an irregular outline and desquamating epithelial cells, in a mouse model expressing a truncated form of ameloblastin.²² These findings highlight the potential role of ameloblastin in the formation and regeneration of the periodontium after disruption of the JE. VPIMDFADPQFPT (Ambn) is an active 13 amino acid cell-adhesion peptide that has recently been identified by deletion analysis and *in vitro* mutagenesis of ameloblastin and specifically binds to extracellular matrix proteins with specific integrin binding functions.²³ Thus, Beyeler et al. suggested that this peptide could be used to promote cell adhesion; however, to date, it has been studied neither to enhance attachment of regenerated tissues to synthetic devices nor, specifically, to induce formation of hemidesmosomes.

Therefore, we hypothesized that bi-peptide coatings with Lam and Ambn peptides will enhance HDs formation and keratinocyte's proliferation, in comparison to mono-peptide coatings and non-coated Ti surfaces. We have proven that stable and robust biofunctional multi-peptide coatings on titanium can be achieved using a silanization route and proper modification of the active peptides.²⁴ Our long-term aim is multifunctionalizing the dental implant surface to enhance the per-mucosal seal and thereby prevent peri-implantitis.

2. MATERIALS AND METHODS

2.1 Peptide Coatings

2.1.1 Peptides—All peptides were produced (purity >98%) by solid-phase peptide synthesis (AAPTec, Louisville, KY). N-terminus of Lam and Ambn peptides was modified with three spacing glycines plus two and three lysines, respectively, to covalently bond the peptides with the silanized titanium surfaces. The lysines also balanced overall size, charge and hydrophilicity between the two peptides to hinder competing interactions with the charged titanium surface during co-immobilization.²⁵ The peptide sequences were Lam, NH₂-KKGGGPPFLMLLKGSTRFC and Ambn, NH₂-KKKGGGVPIMDFADPQFPT. The GL13K-NH₂ peptide, NH₂-GKIIKLKASLKLL-CONH₂ was used as a control because GL13K-NH₂ coatings are highly hydrophobic and lack bioactivity on mammalian cells.²⁶ Fluorescently-labeled peptides were synthesized by conjugating TAMRA-red probe to Lam and 5,6-FAM-green probe to Ambn and GL13K-NH₂; NH₂-KKGGGPPFLMLLKGSTRFC-K-5-TAMRA, Lam-TAMRA; NH₂-KKKGGGVPIMDFADPQFPT-TK-5,6-FAM; Ambn-FAM; and NH₂-GKIIKLKASLKLL-TK-5,6-FAM, GL13K-FAM.

2. 1. 2 Titanium surface preparation—Commercially Pure Titanium Grade II discs (McMaster-Carr, Robbinsville, NJ) were punched, ground and polished, activated by etching (5M-NaOH (Sigma-Aldrich St. Louis, MO), 60°C, overnight), and cleaned with solvents (deionized water (DI, Mili-Q Plus), isopropanol, and acetone (eTi)).

2. 1. 3 Silanization—Silanization of eTi was performed as previously described.²⁴ Briefly, in a N₂-saturated atmosphere, discs were immersed in pentane containing 0.05M N,N-diisopropylethylamine and 0.5M 3-chloropropyl-(triethoxy)silane (CPTES) (all chemicals Sigma-Aldrich, St. Louis, MO); and cleaned with solvents (eTi-Sil).

2.1. 4 Immobilization of peptides—eTi-Sil discs were immersed in 0.1 mM peptide(s) solutions in 0.5mg/ml Na₂CO₃ overnight, and cleaned with solvents. Monopeptide and bi-peptide coatings were fabricated. Bi-peptide coatings were obtained by either sequential or simultaneous immersion of eTi-Sil surfaces in LAM and Ambn peptide solutions.

The following surfaces were tested:

1. eTi - alkaline etched Ti, control
2. eTi-Sil - silanized, control
3. GL13K - coated with GL13K-NH₂, control
4. LAM - coated with Lam
5. AMBN - coated with Ambn
6. LAM+AMBN - sequentially coated with Lam and Ambn
7. AMBN+LAM - sequentially coated with Ambn and Lam
8. LAM/AMBN - simultaneously coated with Lam and Ambn

2. 2 Surface Characterization

2. 2. 1 X-ray photoelectron spectroscopy (XPS)—XPS (SSX-100, Al K α x-ray, 1mm spot-size, 35° take-off angle) was performed to characterize the atomic composition of the treated surfaces. Survey scans (0–1100eV binding energy, 8 scans/sample) were performed at 1eV step-size. Peak fitting and semi-quantification of surface elemental composition were conducted using ESCA 2005 software.

2. 2. 2 Water Contact Angles—DI water contact angles were measured with the sessile-drop (2 μ L) method to assess wettability of the surfaces using a contact angle analyzer (DM-CE1, Kyowa, Japan) with appropriate software (FAMAS, Kyowa, Japan). Contact angles at stability are reported (WCA).

2. 2. 3 Visualization of fluorescent-labeled peptide coatings—Titanium was coated with immobilized fluorescently-labeled peptides, Lam-TAMRA (red), Ambn-FAM (green) and GL13K-FAM (green) following the same protocols described above. All peptides were labeled in their C-terminus. Fluorescence color and intensity of coatings were visualized with a multi-channel fluorescence microscope (Eclipse E800, Nikon, Japan).

2. 2. 4 Mechanical and Thermochemical Stability of the Coatings—All surfaces were mechanically and thermochemically challenged by ultrasonication in deionized water for 2h and subsequent immersion in phosphate buffer solution (PBS, pH 7.4, 37°C) for 8 days.

2. 3 In vitro Cell Experiments

2. 3. 1 Keratinocyte culture—TERT-2/OKF-6 immortalized keratinocytes (BWH Cell Culture and Microscopy Core, Boston, MA) were cultured (37°C, sterile atmosphere of 5% CO₂) in defined keratinocyte serum-free medium (K-SFM(1X)) supplemented with Epidermal Growth factor and Bovine Pituitary Extract (all Thermo Fischer Scientific, Waltham, MA). The medium was changed every two days. Cells were used for all experiments after passage number four.

2. 3. 2 Assessment of Hemidesmosome formation by Immunofluorescence Microscopy—TERT-2/OKF-6 cells (1×10^5) were allowed to attach to the substrates for 24 or 48h and fixed in ice-cold methanol. Cells were incubated in 1% bovine serum albumin (BSA) in PBS with Tween-20 (PBST) to block non-specific binding of the primary antibody. HDs were detected using a primary rabbit polyclonal antibody, targeting human collagen XVII (BP180) (ab28440, Abcam, Cambridge, MA) diluted in 1% BSA in PBST (1:350). Specimens were incubated for 1h in Alexa Fluor 488-conjugated goat anti-rabbit IgG (H+L) secondary antibody (#A-11034, Thermo Fischer Scientific, Waltham, MA, USA) in 1% BSA in PBST (1:500). DAPI staining (4',6-Diamidino-2-Phenylindole, Dihydrochloride, D9542, Sigma-Aldrich, St. Louis, MO) was used to visualize nucleic acids. Images were acquired with fluorescence microscope using the SPOT advance software (Spot Imaging, Sterling Heights, MI) and analyzed and processed with ImageJ (ImageJ 1.49v, NIH, Bethesda, MD) under equal gamma, brightness and time exposure. Five fields per sample were examined. Total fluorescent signal intensity in each field was quantified after proper adjustment for background signal. Controls experiments were performed for determining background immunostaining reactivity of the surfaces in the absence of cells and for assessing non-specific binding of the primary antibody with IgG isotope control in the same concentration as used for test experiments. Staining intensity in these control experiments was negligible.

2. 3. 3 Cell proliferation assay—Keratinocyte proliferation was assessed at 1, 24 or 48h in culture via quantification of DAPI-positive nuclei. Analysis was performed with ImageJ under equal gamma, brightness and time exposure, in a total of 5 fields per sample.

2. 4 Statistical analysis

All experiments were performed at least in triplicate. Results are reported as Mean $\pm$ Standard Deviation (SD). For cell proliferation and HDs formation, one-way ANOVA was carried out for group comparisons. Bonferroni's method was used to adjust for multiple comparisons; thus, for comparison with each control and for pairwise comparison among all the five experiment groups, cut-off p-values were set at 0.01 and 0.005, respectively (SAS v9.3, SAS Institute, Cary, NC).

3. RESULTS

3. 1 Immobilization and Stability of Peptide Coatings

Mono- and bi-peptide biofunctionalized titanium surfaces were obtained through a three-step method using CPTES as coupling agent. WCA (Figure 1), elemental composition determined by XPS (Table 1, Figures S1–S3), and fluorescent visualization (Figure 2) of peptide coated and control surfaces, before and after mechanical and thermochemical challenges provided evidence of the immobilization and stability of the peptides.

WCA on eTi were very low indicating high hydrophilicity due to increased surface area after etching and formation of abundant hydroxyl groups.²⁶ Silanization of the hydroxyl-activated eTi surfaces (eTi-Sil) significantly increased WCA as the organosilane CPTES contains an alkyl chain in the core of the molecule. Chlorine detection on eTi-Sil surfaces confirmed the presence of the silane molecules and thus, successful silanization of the surfaces. GL13K-NH₂-coated control surfaces (GL13K) were notably hydrophobic and after challenges maintained stable WCA, as previously reported.²⁶ The formation of the peptide coating was further confirmed by detection of Nitrogen (N1s peak) on GL13K surfaces from the aminoacid backbone of the peptide molecules. %C also increased in GL13K coated surfaces compared to eTi-Sil surfaces. After mechanical and thermochemical challenges the %N and %C remained notably high, indicating stability of the peptide coating. Finally, GL13K-FAM peptides displayed a homogeneous fluorescent signal on GL13K surfaces, both before and after challenges.

Since our control surfaces confirmed the effectiveness of this chemical route to obtain peptide coatings on titanium, we subsequently investigated the formation of the new biofunctional coatings of Lam and/or Ambn peptides. All surfaces coated with Lam and Ambn were more hydrophilic than eTi-Sil and had increasing WCA after mechanical and thermochemical challenges, except for LAM+AMBN. XPS data and fluorescent visualization for all coatings further confirmed the presence of Lam and Ambn peptides and their stability after challenges. %N and %C increased after peptide(s) immobilization in all peptide-coated surfaces. AMBN surfaces had notably lower %N and %C than surfaces coated with Lam, alone or in combination with Ambn (Table 1). Despite this finding confirming a lower amount of Ambn peptides on Ti surfaces, fluorescently-labeled coatings were found homogeneous for all groups, including AMBN. Merged yellow-orange fluorescent signal of the bi-peptide coated surfaces indicated successful co-immobilization of Lam-TAMRA and Ambn-FAM peptides, following all different methods for sequential and simultaneous exposure of the surfaces to the two peptides.

All peptide-coated surfaces, including AMBN surfaces, presented higher %N and %C than eTi and eTi-Sil surfaces after mechanical and thermochemical challenges. However, a continuous reduction of %N and %C was detected after application of the sequential challenges, which was correlated with the increasing values of WCA (Figure 1). Fluorescent images of the challenged surfaces also suggested substantial amounts of Lam-TAMRA and Ambn-FAM retained peptides (Figure 2). Overall, the reported results corroborated that all peptide coatings were sufficiently retained under these experimental conditions.

Notably, the amount of peptides retained after immobilization, as well as after the mechanical and thermochemical challenges varied depending on the specific peptide and the sequence used for co-immobilizing peptides, as shown in Table 1 for %N and %C.

3. 2 Keratinocyte Response to Biofunctional Peptide Coatings

We assessed TERT-2/OKF-6 keratinocyte proliferation and HDs formation *in vitro* to evaluate potential effects of peptide-coated surfaces on formation of an effective soft-tissue seal around dental implants. Cell proliferation was assessed after 1, 24 and 48h in culture (Figure 3). All Lam coated surfaces (LAM, LAM+AMBN, AMBN+LAM, LAM/AMBN) enhanced keratinocyte proliferation after just 1h in culture, compared to control surfaces (eTi, eTi-Sil, GL13K). After 24 and 48h, all biofunctional peptide coatings presented increased keratinocyte proliferation compared to controls, indicating that both Lam and Ambn peptides exert stimulatory effects on proliferation of keratinocytes. Over time, cell numbers increased in all tested surfaces, except for LAM+AMBN between 24 and 48h in culture (Figure 3B). Indeed, cell proliferation between 24 and 48h was notably higher on monopeptide than on bi-peptide coatings so that after 48h only monopeptide coated LAM and AMBN surfaces had significantly higher keratinocyte proliferation than all controls (Figure 3A).

HDs formation by TERT-2/OKF-6 was assessed on treated surfaces after 24 and 48h in culture (Figure 4). All surfaces coated with Lam peptides stimulated HDs formation; LAM surfaces showed significantly higher HDs formation after 24h than controls (Figure 4A). After 48h, all Lam and Ambn peptide coated surfaces significantly increased HDs formation when compared to controls (Figure 4B). Coating with both peptides had a stimulatory trend on HDs formation as more HDs were formed on all bi-peptide coatings than on all monopeptide coatings. Ti surfaces coated with initial exposure to Ambn peptides, followed by exposure to Lam peptides (AMBN+LAM), had significantly higher HDs formation than monopeptide coatings after 48h in culture. No significant differences were detected between the three co-immobilized peptide coatings ($p>0.05$). The effects of the coatings on HDs formation were also qualitatively assessed by fluorescence visualization of HDs and keratinocytes (Figure S4).

4. DISCUSSION

We aimed producing and evaluating new peptide coatings with biofunctionality to induce fast and effective formation of peri-implant mucosal seal. Since laminin 332 is involved during JE formation and establishment of its attachment to natural teeth, and ameloblastin participates in regeneration of the periodontium, we hypothesized that coatings incorporating both peptides would have synergistic effects on keratinocyte responses *in vitro*. We assessed the effects of these coatings on keratinocyte proliferation and HDs formation. In addition, we investigated the efficacy of using silanization to immobilize Lam and Ambn peptides on titanium, as well as the mechanical and thermochemical stability of the peptide coatings.

In combination, results for WCA, elemental composition and fluorescent visualization of the coatings demonstrated that peptides were effectively immobilized on all monopeptide and

bi-peptide coatings. Moreover, all coatings were stable as they retained significant portions of the peptides after 2h sonication in water, followed by 8d of immersion in PBS at physiological temperature and pH.

The %N for LAM and AMBN coatings was higher than for the control GL13K-NH₂ coating, except for AMBN surfaces. This confirms the effective immobilization of these peptides when using previously reported CPTES-silanization method. eTi-Sil surfaces are negatively charged²⁷ and hydrophilic (Figure 1). Effectiveness of peptide immobilization on titanium using silanes increases when surface and peptides have opposite charges.²⁵ Thus, by adding lysines at the N-terminus of our peptides, we achieved an overall positive charge. Additionally, we aimed balancing length/molecular weight and hydrophilicity to minimize competition between Lam and Ambn peptides when reacting with the eTi-Sil surfaces. Thus, Lam and Ambn have 19 aminoacids each, with pI 10.94 and 9.63, and +4 and +1 theoretical charge at neutral pH, respectively; however, Ambn is significantly more hydrophilic than Lam as it includes two aspartates. We effectively co-immobilized the two peptides on the same surface, regardless of the specific sequence used to obtain the bi-peptide coatings. This approach also provided stability to the two co-immobilized peptides, as confirmed by the homogeneous orange/yellow signal on challenged LAM-TAMRA and AMBN-FAM surfaces. The notably lower amount of immobilized peptides in AMBN surfaces might be related to a lower electrostatic attraction of the Ambn peptides to eTi-Sil surfaces compared to Lam peptides. However, these surfaces showed significant fluorescent signal before and after challenges and induced significant effects on keratinocytes response compared to control surfaces.

Notably, the tethering of our Lam peptides to the titanium surfaces using CPTES as linker was not site-specific. Lam peptides contained a lysine close to its C-terminus that competed with the added lysines at the N-terminus and the N-terminal α -amine for reacting to the silane molecules. This lack of chemoselectivity and thus, site-specific reactivity can influence orientation and 3-dimensional conformation of the anchored Lam peptides. This is relevant as bioactivity of peptides is generally dependent on achieving specific conformations.²⁸ Others have developed chemoselective routes for site-specific reactivity of peptides and studied and validated the specific effects that conformation and/or orientation of the peptides have on its biological activity. Examples of chemical routes for site-specific anchoring of peptides include Michael addition of cysteine-containing peptides to maleimide-modified substrates,^{29, 30} 'click' chemistry,³¹ native chemical ligation,³² hydroxylane derivatization of unprotected peptides that can specifically react with silanol-containing surfaces,³³ and specific catechol derivatization of the N-terminal α -amine.³⁴ The latter has been recently developed and it is especially appropriate for site-specific tethering of the Lam peptides used here as catechol specifically reacts with the N-terminal α -amine, avoiding the ϵ -amine group in lysines. In our work, although we did not apply a chemoselective route and did not achieve site-specific tethering of the Lam peptides, the two lysines added to their N-terminus in combination with the N-terminal α -amine provided a local electropositive pole. Electrostatic attraction between peptides and silanized titanium surfaces effectively affects peptides covalent attachment²⁵; which in turn, might favor reactions between the amines near the N-terminus of the Lam peptides and CPTES molecules. Our LAM coatings increased formation of HDs by keratinocytes in comparison

to control surfaces indicating that a chemoselective route for tethering the peptides might not be critical to bioactivate the surface with these peptides. However, future work should focus on improving the biological activity of the peptides coatings by applying chemoselective routes to tether the Lam peptides to the titanium surfaces and therefore have control over their specific orientation and conformation.

Proliferation of keratinocytes was stimulated in all biofunctional peptide-coated surfaces compared to control surfaces. This result confirms a significant role for the Lam peptide in promoting keratinocyte growth³⁵, but contradicts previous reports on the effects of Ambn peptide on downregulation of epithelial proliferation.³⁶ Moreover, the peptide coatings stimulated HDs formation by keratinocytes. Our hypothesis was validated as coatings with both Lam and Ambn peptides synergistically enhanced HDs formation with AMBN+LAM surfaces inducing significantly higher HDs formation than all mono-peptide-coated and control surfaces. Although previous investigations have shown that ameloblastin is involved in processes other than amelogenesis^{21, 37, 38}, the present investigation is the first to examine the effect of the Ambn peptide on HDs formation. We validated our hypothesis using commercially pure titanium Grade II surfaces. Most of the commercial dental implants and metallic abutments are made of titanium Grade IV or other titanium alloys.³⁹ We have previously demonstrated that homogeneous and robust peptide coatings can also be obtained on titanium Grade IV, other titanium alloys and zirconium oxide using the same silanization route than the one reported here.^{40, 41}

All surfaces with bi-peptide coatings showed a pronounced increase in cell numbers at early time points (1h, 24h), while the mono-peptide-coated surfaces had the highest cell numbers among all tested surfaces at 48h. Interestingly, the bi-peptide coated surfaces induced stronger effects on HDs formation at 48h than the mono-peptide-coated surfaces. Confirming these trends, LAM+AMBN were the only surfaces that did not increase keratinocyte numbers between 24h and 48h of culture, but had of the highest HDs values at 24h. Keratinocytes have two inter-changeable modes of proliferation, the balanced and the expanding mode, and switching from the expanding to the balanced mode occurs at keratinocyte confluence. Upon that switch, keratinocytes differentiate.⁴² Thus, keratinocytes on bi-peptide coatings were most likely to be in the expanding mode at early time points and switch to the balanced mode sometime between 24h and 48h in culture; i.e., keratinocyte critical confluence was reached earlier on these surfaces. This, in turn might accelerate HDs formation.

Together, these findings suggest that the effects of stable coatings made with both Lam and Ambn peptides resulted in an acceleration of the processes for both keratinocyte proliferation and HDs formation.

5. CONCLUSIONS

Laminin and ameloblastin peptides' coatings accelerated keratinocyte proliferation and, most significantly, hemidesmosome formation in vitro. Thus, the laminin and ameloblastin bi-peptide coatings have potential to improve the per-mucosal seal around dental implants to address the critical need of preventing peri-implantitis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

Ambn	VPIMDFADPQFPT ameloblastin peptide
CPTES	3-chloropropyl-(triethoxy)silane
HDs	hemidesmosomes
JE	Junctional epithelium
Lam	PPFLMLLKGSTRFC laminin 332 peptide
Ti	Titanium
XPS	X-ray photoelectron spectroscopy

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Figure 1.

Water contact angles for all control and peptide-coated titanium surfaces before challenges and after mechanical (ultrasonication in water for 2h) and thermochemical (8 days in PBS, 37°C, pH=7.4) stability challenges. Error bars represent Standard Deviations.

Figure 2.

Visualization of fluorescently-labeled peptide coatings and control surfaces. A. Before challenge; B. After ultrasonication for 2h in water; and C. After ultrasonication for 2h in water and 8 days of immersion in PBS at 37°C, pH=7.4. Immobilized fluorescently-labeled peptides were Lam-TAMRA (red), Ambn-FAM (green) and GL13K-FAM (green).

Figure 3. Proliferation of keratinocytes on all control and biofunctional peptide-coated Ti surfaces. A. Number of keratinocyte cells (TERT-2/OKF-6) after 48h in culture. Error bars represent Standard Deviations. B. Keratinocyte proliferation over time for all tested surfaces in culture.

Figure 4. HDs formation by keratinocytes (TERT-2/OKF-6) on all control and biofunctional peptide-coated Ti surfaces. A. After 24h in culture and B. After 48h in culture. Error bars represent Standard Deviations.

Table 1

Average elemental composition (%) determined by XPS for all control and biofunctional peptide-coated surfaces before and after mechanical (ultrasonication in water for 2h) and thermochemical (8 days in PBS, 37°C, pH=7.4) stability challenges. sd: Standard Deviation.

Treatment	Surface	Cl1s	N1s	O1s	Si2p	Cl2p	Ti2p	Na1s	N/Ti	C/Ti
Pre Challenges	eTi	9.00	0.00	60.40	0.00	0.53	22.37	7.40	0.00	0.41
	SD	3.48	0.00	7.33	0.00	0.61	0.90	4.03	0.00	0.17
	eTi-Sil	16.17	0.00	54.90	1.50	3.10	17.80	5.97	0.00	0.91
	SD	1.39	0.00	3.18	0.30	0.52	1.20	5.17	0.00	0.13
	GL13K	40.43	10.43	37.53	0.00	0.13	11.43	0.00	0.91	3.54
	SD	1.70	0.35	1.68	0.00	0.23	0.67	0.00	0.06	0.17
	LAM	51.33	12.87	30.07	0.00	0.00	5.77	0.00	2.32	9.13
	SD	1.55	1.40	1.98	0.00	0.00	1.12	0.00	0.67	1.78
	AMBN	23.63	4.27	54.10	0.00	0.00	18.00	0.00	0.24	1.35
	SD	2.75	0.76	1.39	0.00	0.00	2.44	0.00	0.07	0.36
	LAM + AMBN	52.93	13.13	28.13	0.00	0.30	4.90	0.00	2.74	11.27
	SD	1.88	1.86	0.45	0.00	0.52	1.15	0.00	0.49	3.02
	AMBN + LAM	53.30	12.93	27.97	0.00	0.00	5.60	0.00	2.31	9.52
	SD	1.25	0.80	1.79	0.00	0.00	0.20	0.00	0.13	0.29
Post Mechanical Challenge	LAM/AMBN	49.40	14.10	29.47	0.60	0.13	5.90	0.00	2.42	8.44
	SD	1.25	0.92	1.63	1.04	0.23	0.62	0.00	0.40	0.96
	eTi	16.80	0.00	56.00	0.00	0.00	17.10	10.07	0.00	0.99
	SD	1.31	0.00	2.14	0.00	0.00	0.92	1.29	0.00	0.12
	eTi-Sil	16.13	0.00	52.70	2.37	2.53	18.03	8.23	0.00	0.89
	SD	1.78	0.00	1.30	0.21	0.40	0.06	0.57	0.00	0.10
	GL13K	44.03	9.50	37.07	0.00	0.00	9.37	0.00	1.02	4.74
	SD	1.27	0.35	0.25	0.00	0.00	0.84	0.00	0.08	0.59
	LAM	51.93	11.63	31.13	0.00	0.27	5.03	0.00	2.31	10.32
	SD	1.58	0.60	2.35	0.00	0.46	0.15	0.00	0.15	0.32
	AMBN	23.10	4.07	55.57	0.00	0.00	17.30	0.00	0.24	1.36
	SD	2.50	0.50	2.49	0.00	0.00	2.27	0.00	0.06	0.28
	LAM + AMBN	49.73	10.57	32.53	0.00	0.00	6.70	0.00	1.59	7.46

Treatment	Surface	C1s	N1s	O1s	Si2p	Cl2p	Ti2p	Na1s	N/Ti	C/Ti
Post Chemical Challenge	SD	0.29	0.49	0.21	0.00	0.00	0.61	0.00	0.21	0.63
	AMBN + LAM	47.33	9.80	34.97	0.00	0.00	7.70	0.00	1.52	7.09
	SD	5.23	2.43	4.36	0.00	0.00	3.49	0.00	0.81	3.15
	LAM/AMBN	50.43	11.07	32.50	0.00	0.00	6.03	0.00	1.87	8.48
	SD	1.14	0.85	2.26	0.00	0.00	0.90	0.00	0.34	1.24
	eTi	10.83	0.00	57.77	0.00	0.00	20.13	11.17	0.00	0.54
	SD	0.95	0.00	1.15	0.00	0.00	0.51	0.61	0.00	0.03
	eTi-Sil	16.07	0.00	53.80	0.00	0.70	18.70	10.43	0.00	0.86
	SD	2.47	0.00	1.15	0.00	0.62	0.26	2.11	0.00	0.14
	GL13K	40.63	8.97	38.97	0.00	0.00	11.40	0.00	0.79	3.57
	SD	0.99	0.90	1.26	0.00	0.00	0.52	0.00	0.12	0.23
	LAM	46.10	10.50	35.13	0.00	0.00	8.27	0.00	1.28	5.62
	SD	1.35	0.30	0.92	0.00	0.00	0.81	0.00	0.17	0.72
	AMBN	16.43	1.37	61.43	0.00	0.00	20.73	0.00	0.07	0.79
	SD	3.64	1.52	3.10	0.00	0.00	0.15	0.00	0.07	0.18
	LAM + AMBN	49.37	8.63	34.03	0.00	0.00	8.00	0.00	1.08	6.18
	SD	2.78	1.61	1.40	0.00	0.00	0.26	0.00	0.17	0.54
	AMBN + LAM	44.47	9.57	36.60	0.00	0.00	9.13	0.00	1.05	4.89
	SD	1.95	0.60	1.65	0.00	0.00	0.71	0.00	0.13	0.50
	LAM/AMBN	48.07	9.43	34.47	0.00	0.00	8.00	0.00	1.18	6.01
	SD	0.70	1.33	0.84	0.00	0.00	0.17	0.00	0.14	0.21