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Enhanced migration and adhesion protein expression by polyethylene glycol 4-modified SVVYGLR peptide in an *in vitro* human gingival fibroblast wound healing model

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ABSTRACT

Introduction. This study was aimed at exploring the effect of four units of polyethylene glycol (PEG4)-modified Ser-Val-Val-Tyr-Gly-Leu-Arg (SVVYGLR) peptide (SV peptide) on the proliferative potential and migrative capability of human gingival fibroblasts (HGFs) and the activation of adhesion-related proteins.

Materials and methods. PEG4-SV peptide was synthesised using peptide solid phase synthesis. The proliferative response of HGFs to varying concentrations of PEG4-SV peptide was quantitatively evaluated using a Cell Counting Kit-8 (CCK-8) assay. The migratory capacity of HGFs in response to PEG4-SV peptide treatment was evaluated using an *in vitro*

wound healing assay. The expression levels of adhesion-related genes, including collagen type I (COL-1), vinculin (VCL), focal adhesion kinase (FAK), and integrin β1(ITGB1), were quantitatively analysed using qRT-PCR. To assess the aforementioned adhesion-related proteins, immunofluorescence and Western blotting were performed.

Results. Primary HGFs were isolated through enzymatic digestion using dispase, and subsequently characterised by positive immunoreactivity for both vimentin and CD90 (Thy-1) markers. Compared to the control group, PEG4-SV at concentrations of 10, 20, and 40 μg/mL significantly promoted the proliferation of HGFs. The wound area in the SV group exhibited a significantly smaller size in the monolayer cell culture at 24 and 48 h. The expression of adhesion-related genes and proteins (collagen type I, vinculin, FAK and integrin β1), were significantly upregulated after treatment with 20 μg/mL PEG4-SV.

Conclusions. These results demonstrate that PEG4-SV peptide may have the ability to promote soft tissue healing around an implant surface and form tight soft tissue sealing on the transmucosal part of the implant.

Keywords: PEG4-SV peptide; human gingival fibroblasts; cell proliferation; cell migration; adhesion genes and proteins

Introduction

Dental implants are a widely-used replacement for missing teeth [1]. The mucosal component around dental implants has a special feature that allows it to penetrate soft tissue. The stability of an implant relies on healthy gingival tissue integration around the implant surface [2]. Histologically, the peri-implant soft tissue seal, composed of stratified epithelium and underlying connective tissue, forms an essential biological barrier against oral microbial colonisation. The breakdown of this barrier can trigger persistent inflammatory responses, potentially leading to peri-implantitis and subsequent implant failure [3].

Human gingival fibroblasts (HGFs), as the predominant cellular constituents within the peri-implant soft tissue seal, play a pivotal role in extracellular matrix (ECM) remodelling through their synthetic capacity to produce collagen and other essential ECM components. Following implant surgery, collagen fibres form after 4−6 weeks, and healthy connective tissue sealing

forms in 6–12 weeks [4]. In contrast to periodontal ligaments, which are directly fixed in the tooth, the morphology of collagenous fibres in soft tissues is mostly parallel to the implant abutment surfaces [5]. Furthermore, the proportion of HGFs in the peri-implant soft tissue is only 3%, significantly lower than the 15% cellular composition found in healthy periodontal tissue [5]. Implants require long-term stability in order to establish a quick and strong connective tissue attachment crossing the mucosal region. In order to enhance connective tissue integration, the early establishment of direct fibre junctions on implants relies on upregulating the activity of HGFs and enhancing the secretion of integrins [6].

Modified titanium materials exhibit biological properties in addition to their inherent strengths, indicating a new direction for implant surface modification. The commonly used bioactive molecules are the ligands of integrins in the ECM, such as laminin 332 [7], fibronectin [8], collagen [9], and other peptides [10].

Synthetic peptides are the most attractive component of bioactive materials because they encapsulate the topological, chemical, and biological properties of natural proteins. They can interact directly with cells to promote protease remodelling and resolution [11–13]. Among them, ECM-derived peptides have been the focus of metal surface modification [14–16].

During wound healing, the components of the ECM play a key role in tissue regeneration [17, 18]. Osteopontin (OPN) is a component of the extracellular matrix and is found in various cells [19]. It has a role in tissue repair and displays angiogenic activity [19]. It has been shown that OPN exposed to the 7-amino acid sequence Ser-Val-Val-Tyr-Gly-Leu-Arg (SVVYGLR) at the N-terminus after thrombin cleavage exhibits strong angiogenic activity [20, 21] SVVYGLR peptide (SV peptide) can bind with α4β1, α9β1, and α4β7 integrins to promote adhesion, migration, and proliferation in endothelial cells [22]. SV peptide is more effective than vascular endothelial growth factor (VEGF), which can promote vascularisation and regulate osteoblast function [23]. Tanaka *et al.* [24] showed that SV peptide can increase the migration and motility of oral mucosal cells. Animal experiments have shown that injection of SV peptide can promote wound contraction to accelerate oral mucosal wound healing [24]. By promoting fibroblast differentiation into alpha-smooth muscle actin (α-SMA) positive myoblasts through transforming growth factor-β (TGF-β)/Smad signalling pathway, SV

peptide activates vascular endothelial cells and smooth muscle cells to regulate angiogenesis [25]. During soft tissue healing, the interplay between integrins and TGF-β is essential for mediating the progression of fibrosis [26]. Specifically, the expression of integrins such as **□1β1 and □2β1 {please clarify?}** upregulates TGF-β, which subsequently promotes collagen reconstruction and myofibroblast-mediated tissue contraction [26].

SV peptide can promote the proliferation of skin and mucosa fibroblasts, collagen synthesis and secretion in oral soft tissue trauma. Although the composition of skin and mucosa is relatively similar to that of the gingiva, there are many differences in terms of oral soft tissue healing methods and implant soft tissue integration. Therefore, it has not yet been demonstrated whether SV peptide can up-regulate the activity of fibroblasts and improve the expression of integrins to support early establishment of direct fibril connection.

This study aimed to explore the influence of polyethylene glycol (PEG)-modified SV peptide on the proliferation and migration of HGFs, and the activation of adhesion-related proteins to provide insights for preparing biological implant coating and soft tissue healing.

Materials and methods

Synthesis of peptide

To improve the solubility and stability of SV peptide, we attached PEG4 which contains four units of PEG to the **N terminompany {N-terminus?}**, with a purity of > 95% determined by mass spectrometry. Peptide freeze-dried powder was refrigerated at –20°C. The PEG4-SV peptide freeze-dried powder was centrifuged at 1,000 revolutions per minute for 1 min. Subsequently, the residual powder of the tube wall was gathered at the bottom of the tube, 1 mL phosphate-buffered saline (PBS) was added, and peptide mother liquor with a concentration of 4 mg/mL was prepared. After being fully mixed, the liquid from the PEG4-SV peptide was filtered and sterilised using a 0.22 μm needle-tube filter membrane, and then stored at 4°C for one month.

Cell culture of human gingival fibroblast

Healthy gingival tissues were obtained from three donors (one man and two women; mean age 12.3 ± 0.9 years, SD) from whom six first premolars were extracted for orthodontic

treatment with written consent. Next, fresh tissues were rinsed with PBS containing a 10% penicillin–streptomycin solution (Gibco, Waltham, MA, USA) and placed in dispase (4 mg/mL; Biosharp, Hefei, Anhui, China) in a 37℃ water bath for 4 h. After gingival connective tissue was easily separated from epithelial tissue, it was collected and cut into 1 mm^3 pieces, dispersed into 25 cm^2 culture flasks, and incubated in Dulbecco's modified Eagle medium (DMEM; Gibco) supplemented with 10% foetal bovine serum (FBS; ZETA USA, Philadelphia, PA, USA) in a cell incubator with 5% CO_2 at 37°C. By using 0.25% trypsin (Solarbio, Beijing, China) for digestion, cells were passaged when they grew nearly confluent. The cells between passages 3 to 6 were used in this research.

Identification of HGFs

To identify HGFs, immunofluorescence analysis was used to verify the presence of vimentin and CD90 (Thy-1) markers which are mostly expressed in fibroblasts [8]. Coverslips with cells were placed in a 24-well plate, on which 1×10^5 cells/mL were seeded and cultured for two days. Then the cell slippers were rinsed with PBS and fixed with 4% paraformaldehyde (PFA). After permeabilisation with 0.25% Triton X-100 (Solarbio, Beijing, China), the samples were blocked by 5% bovine serum albumin (BSA, Servicebio, Wuhan, Hubei, China) for 1 h. Then the samples were incubated with mouse anti-vimentin antibody (1:500, Servicebio GB12192, China) and rabbit anti-CD90 antibody (1:200, Servicebio GB113753, China) overnight at 4°C. The next day, the cells were washed with PBS and incubated with secondary antibody, including Alexa Fluor 488-labelled goat anti-mouse IgG (1:2,000, Servicebio GB25303, China) and CY3-labelled goat anti-Rabbit IgG (1:2,000, Servicebio G21301, China) for 1 h. Finally, 4',6-diamidino-2-phenylindole (DAPI; Servicebio, China) was used to stain the nucleus. All samples were observed using an ECLIPSE C1 fluorescence microscope (Nikon, Tokyo, Japan).

Cell viability and proliferation

The effect of PEG4-SV peptide on the proliferation of HGFs was evaluated using a cell counting kit (CCK-8; ZETA). After seeding 1×10^4 HGFs into 96-well plates for one day, cells

were moved to a culture medium containing various concentrations of PEG4-SV peptide (0, 1, 5, 10, 20, 40, and 80 μg/mL). After days 1, 2, and 3, the wells were rinsed in PBS and incubated in 100 μL of DMEM containing a 10% CCK-8 solution for 1 h. Finally, optical density (OD) values at 450 nm were tested using a microplate reader (KHB, China). The OD value determined the proliferation of HGFs.

Cell migration

The influence of PEG4-SV peptide on cell migration was analysed using a wound healing test. HGFs were seeded at a density of 5×10^5 cells/well into 6-well plates and incubated until they reached 100% confluence. Five horizontal parallel lines were made on the bottom of the plate to mark the captured view. A 100-μL micropipette tip was used to make a vertical wound on the well plate. The wells were then washed clean of debris and cultured for another 24 and 48 h in a serum-free medium with different concentrations of PEG4-SV peptide (0, 10, 20, and 40 μg/mL). At marked observation points, an image of the wound size was captured at a 100× magnification under a light microscope.

Adhesion-related gene expression

The expression of adhesion-related and ECM components genes was investigated using a real time quantitative polymerase chain reaction (RT-qPCR). HGFs were seeded in 6-well plates at a density of 2×10^5 and incubated for 24 h. Subsequently, the cells were cultured for another 72 h in a cell medium containing different concentrations of PEG4-SV peptide (0, 10, 20, and 40 μg/mL). Total RNA was extracted using an EZ-press RNA Purification Kit (B0004D, EZBioscience, Roseville, MN, USA) according to the manufacturer's instructions. The concentration and quality of RNA was tested using a NanoDrop spectrophotometer (Thermo Fisher, Waltham, MA, USA). Using a Colour Reverse Transcription Kit RNA purification kit (A0010CGQ, EZBioscience), the complementary DNA was synthesised through reverse

transcription. Using a Colour SYBR Green qPCR Master Mix (A0012-R2, EZBioscience), the target gene expression was quantified. Specific gene primers, including collagen type 1 (COL-1), focal adhesion kinase (FAK), vinculin (VCL), and integrin β1 (ITGB1), were synthesised (Qinke, Changsha, Hunan, China) and are set out in Table 1, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serving as the housekeeping gene. The reaction was carried out for 40 cycles using the following programmed parameters: 95°C for 10 minutes; 95°C for 2 seconds; 60°C for 20 seconds, and 70°C for 10 seconds.

Immunofluorescence

Specific immunofluorescences for COL-1, FAK, VCL and ITGB1 were used to measure the level of focal adhesion (FA) and the secretion of ECM during cell adhesion. HGFs were seeded on coverslips in 24-well plates at a density of 5×10^4 cells/well and cultured for 24 h. Subsequently, the cells were cultured for another 48 h in a medium with different concentrations of PEG4-SV peptide (0 and 20 μg/mL). The cells were fixed in 4% PFA and permeabilised with 0.1% Triton X-100 for 10 min. After blocking with 10% goat serum (EpiZyme, Shenzhen, Guangdong, China), cells were incubated with antibodies against COL-1 (1:100, ab34710, Abcam, Cambridge, UK), FAK (1:100, ET1602, Huabio, Shenzhen, Guangdong, China), VCL (1:100, ab129002, Abcam), and ITGB1 (1:100, Servicebio, China) at 4°C overnight. After washing, DyLight 647-conjugated goat anti-rabbit (Abclonal, China) was used for incubating cells in the dark for 1 h. For actin staining, cells were incubated by Alexa Fluor488 phalloidin (Solarbio, China) for 1 h. DAPI (Solarbio, China) was used to stain the nuclei for 5 min. The images of stained cells were observed using a confocal laser-scanning microscope (CLSM, ZEISS, Oberkochen, Germany).

Western blotting

The effect of PEG4-SV peptide on adhesion-related proteins was analysed by Western blotting. In 6-well plates, 1×10^5 cells were seeded and cultured for one day. Subsequently, the cells were cultured for another 72 h in a cell culture medium with different concentrations of PEG4-SV peptide (0 and 20 μg/mL). The cells were then lysed with a

radioimmunoprecipitation assay (RIPA), obtained using 1% protease inhibitor (EpiZyme), collected with a scraper, and the lysates were collected after centrifugation. After counting the concentration of total cell proteins, its samples (containing 20 μg protein) were isolated on 7.5% SDS-PAGE gels (EpiZyme), then moved to 0.45 μm PVDF membranes (EpiZyme). PVDF membranes were immediately blocked by Quick Block buffer (EpiZyme) for 15 min and incubated with primary antibodies, including COL-1A1 (1:1,000, Huabio), FAK (1:1,000, Huabio), VCL (1:1,000, Abcam), ITGB1 (1:5,000, Servicebio), and GAPDH (1:20,000, Huabio) at 4°C overnight. PVDF membranes were incubated with HRP-conjugated secondary antibodies (1:5,000, EpiZyme) for 1 h. An ECL chemiluminescent kit (EpiZyme) was used to visualise the protein bands, which were captured by a ChemiDoc Touch imaging system (Tanon-5200, China).

Statistical analysis

Data was analysed using GraphPad software version 8 (GraphPad Software, San Diego, CA, USA). Differences in groups were analysed using analysis of variance (ANOVA). Statistical significance was set at $P < 0.05$.

Results

Identification of human gingival fibroblasts

Primary HGFs were obtained using dispase enzyme. The proliferating cells have typical spindle-shaped morphology (Fig. 1A). Since the markers of vimentin and CD90 (Thy-1) are typically expressed in fibroblasts, their presence was clearly demonstrated by immunofluorescence staining (Figs. 1B, C).

Cell proliferation

Cell proliferation was detected by CCK-8 assay. As shown in Fig. 2, the proliferative activity of HGFs in all groups increased in a time-dependent manner. Significant enhancement of cell proliferation was observed at concentrations of 10, 20, and 40 μg/mL compared to other

groups ($P < 0.05$). On day 3, the **□ □g/mL and 80 □g/mL {please clarify?}** groups grew slowly compared to the control group.

Effects of PEG4-SV on cell migration

The effect of PEG4-SV on HGF migration was determined using a wound-healing assay (Fig. 3A). After culturing for 24 h and 48 h, the healing rate of the control group was lower than that of the PEG4-SV peptide groups, and indicated lower motility (Fig. 3A). After stimulation with PEG4-SV peptide, the wound area on monolayer cells was smaller than that of the control group in 24 h, and cells gradually filled the wound within 48 h (Fig. 3A). However, no significant variation was observed among different concentrations of PEG4-SV peptide groups (Fig. 3B).

Expression of adhesion-related genes

Adhesion-related gene expression levels of HGFs in different groups were analysed by RT-qPCR (Fig. 4). After being cultured for 72 h, the PEG4-SV peptide group (20 and **40 □g/mL**) had higher levels of COL-1, FAK, and VCL mRNAs than the control group. Gene expression of ITGB1 was stimulated significantly by PEG-SV peptide (**20 □g/mL**), while significantly decreased mRNA expression was observed in the **40 □g/mL** group.

Morphological expression of adhesive proteins

Immunofluorescent images exhibited the effect of PEG4-SV peptide on the expression of adhesive proteins (COL-I, VCL, FAK and ITGB1) and actin cytoskeleton. In the PEG4-SV peptide group, adhesion-related proteins exhibited enhanced fluorescence intensity (red) within the cytoplasm of the cells and widely distributed along the cytoskeleton (Figs. 5 A–D). The difference in mean fluorescence intensity was statistically significant (Fig. 5 E). After 48

h, HGFs cultured with PEG4-SV peptide had more distinct spreading and more pronounced filamentous feet compared to the control group (Figs. 5 A–D).

Levels of adhesion-related proteins in HGFs

Consistent with previous experimental results, the levels of adhesion-related proteins in HGFs were studied using Western blotting. The relative content of COL-1A1, FAK, VCL and ITGB1 were enhanced by PEG4-SV peptide (20 μg/mL) compared to the control group (Fig. 6).

Discussion

Following implant surgery, HGFs migrate to the wound site, proliferate and secrete collagen-rich ECM on the implant surface [27]. During this process, the interaction between HGFs and the ECM is crucial. Establishing the functionality of the implant abutment by crosslinking ECM proteins or peptides, including laminin332, fibronectin, type I collagen and arginine-glycine-aspartic acid (RGD) sequences promotes integrin-mediated signal transduction and cell adhesion to the implant surface [28].

Released from the osteopontin by thrombin cleavage, SV peptide is involved in specialised functions including cell migration, attachment, and inflammation [29]. OPN contains various cell-binding domains, including RGD and SVVYGLR [30]. In the SV peptide chain (Ser-Val-Val-Tyr-Gly-Leu-Arg), Val (valine), Tyr (tyrosine), Gly (glycine), and Leu (leucine) are all hydrophobic amino acids, which exhibit low solubility in water.

To improve solubility, PEG modification is straightforward to perform, and can significantly enhance the stability of peptides [31, 32]. However, the choice of modification site is crucial to maintain peptide activity. Studies have shown that these residues (Val164, Tyr165, and Leu167) are essential for binding with integrins α4β1 and α9β1 to mediate cell removal and

adhesion. The residue Arg168 can bind to integrin α9, whereas integrin α4 binds to SVVYGLR [22]. Therefore, to preserve the binding site of the integrin, we modified the N terminal on PEG4 of SVVYGLR using a solid phase synthesis method to address the low solubility and improve the stability of the short chain polypeptide [33]. The purity of the obtained PEG4-SVVYGLR was shown to be 97.47% through the use of analytical high-performance liquid chromatography. This could provide an approach for subsequent studies seeking to covalently immobilise PEG4-SV onto modified metal abutment surfaces.

HGFs can migrate to specific sites of tissue damage and mediate local tissue restoration and regeneration [34]. Tanaka *et al.* [24] revealed that SVVYGLR peptide advanced cell motility and accelerated oral soft tissue wound healing, while SV peptide had no effect on cell proliferation in either fibroblasts or keratinocytes.

However, our current study has shown that the proliferation of HGFs was stimulated by PEG4-SV peptide (10, 20 and 40 μg/mL) treatment groups after being cultured for one, two and three days. We speculate that this was possibly because previous studies used mucosal fibroblasts and keratinised epithelial cells [35], while our study focused on HGFs. Another reason may be the increased stability offered by PEG modification of SV peptide. Through wound healing assay, we also showed that the wound area on the monolayer cell was smaller at 10, 20, and **40 □□g/mL** of the SV peptide group compared to the control group. This is consistent with the CCK-8 assay outcomes, which illustrated the influence of PEG4-SV peptide on migration and proliferation in HGFs.

However, the mechanism of implant soft tissue integration is both similar to, and at the same time different from, that of oral mucosal wound healing. The implant abutment directly faces connective tissue. In this microenvironment, the reaction between connective tissue and metal compounds (such as Ti, TiO_2, and ZrO_2) can have a significant influence on cells [36, 37]. **The cellular component in peri-implant connective tissue is about 2% and 10% which is considered 'scar' tissue rather than a living organ like the periodontal ligament {unclear sentence?}} [38, 39]**. The connective tissue, which has more stroma-like structures and less

cell activity, is sensitive to stimulation from the oral microenvironment. Therefore, the surface modification of implant abutments should focus on promoting cell proliferation, cell migration, and fibroblast adhesion.

Derived from mesenchyme, HGFs play a significant role in the formation of ECM [40]. These cells are essential for synthesis and remodelling of the matrix. Type I collagen constitutes the major component of the matrix and accelerates the subsequent formation of the bioseal [41]. We examined whether the PEG4-SV peptide highly expressed the gene and protein of COL-1 using qRT-PCR, immunofluorescence, and Western blotting methods. Previous studies revealed that the thrombin cleavage of the N-OPN-exposed SVVYGLR motif was possibly related to the differentiation of fibroblasts into α-SMA positive myoblasts by activating the TGF-β/Smad signalling pathway [26]. TGF-β can react with cell receptors located in the cell membrane, triggering the phosphorylation of Smad2 and Smad3, and regulating the expression of target genes such as COL-1 [42, 43]. Through the glycolytic pathway, TGF-β can also stimulate the secretion of various enzymes, which is crucial for collagen synthesis [44].

Integrins mediate cellular anchorage to the extracellular matrix (ECM) through binding to structural components such as collagen, fibronectin, and laminin. This interaction triggers the recruitment of cytoskeletal adaptor proteins (e.g. vinculin, talin, paxillin) and intracellular signalling molecules, initiating the assembly of focal adhesion complexes. These dynamic structures not only strengthen cell-matrix adhesion, but also serve as mechanical transduction hubs [45]. Previous studies have shown that **SV peptide has bonding site {SV peptide bonds ?}** with integrin α4β1, α9β1, α4β7 to mediate cell migration [22]. Our study showed that PEG4-SV peptide, especially at 20 **□□g/mL**, enhanced the gene and protein expression of integrin β1, which plays a crucial role in promoting cellular signalling.

The activation of FAK is a key step in the formation of focal adhesions. Upon integrin-ECM binding, the Tyr397 site of FAK undergoes autophosphorylation, activating downstream signalling pathways, including Rho GTPases [46]. These pathways regulate cytoskeletal reorganisation and cell migration [47]. As actin filaments polymerise and crosslink, focal

adhesion complexes gradually mature and eventually form stable fibrillar adhesions [48]. Focal adhesion complexes are regarded as biochemical signal centres that trigger many signalling proteins and play a vital role in oral mucosal sealing around implant abutments [46, 48, 49]. Moreover, vinculin (VCL) is an major component of focal adhesion complexes, contributing to their stability and regulating the strength of cell-ECM adhesion and cell migration [50, 51]. We found that PEG4-SV peptide stimulated the gene and protein expression of FAK and VCL. Immunofluorescent staining indicated that FAK and VCL were highly expressed in the cells, and that the cytoskeleton displayed a more spread morphology compared to the control group.

Therefore, PEG4-SV peptide enhances the expression of adhesion-related proteins (COL-1, FAK, VCL, and ITGB1), promotes the formation and maturation of FA, and strengthens the signalling hub between HGFs and ECM. This process regulates cell adhesion, migration, proliferation, and differentiation, and facilitates cell movement toward the damaged site, and thereby may promote the integration of soft tissue within the implant surface.

Conclusions

PEG4-SV peptide can significantly enhance HGFs proliferation and migration. Meanwhile, PEG4-SV peptide increases cell adhesion, FA formation, and ECM secretion by stimulating the integrin-mediated signal, suggesting that it may promote soft tissue healing around the implant surface and form tight soft tissue sealing. Due to the many factors affecting soft tissue integration, PEG4-SV peptide modification strategies for metal surfaces should be further studied, with a focus on factors including adhesion stability, slow-release system, and antibacterial properties.

Article information and declarations

Data availability statement

The datasets used and/or analysed during the current study are available from the corresponding authors upon reasonable request.

Ethics statement

This study was conducted in accordance with the Declaration of Helsinki. This study was approved by the Ethics committee of Nanfang Hospital, Southern Medical University (NO. 2022-693). Written informed consent was obtained from the participants or their guardians.

Authors' contribution

Study conception and design: PX, LC, YH, BW. Data collection, analysis, and interpretation: PX, CY. Manuscript writing: PX. Critical revisions for intellectual content: LC, YH, BW. Approval of final version of the manuscript: PX, CY, ZC, LC, YH, BW.

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Conflict of interests

The authors declare no conflict of interest.

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Table 1. Primer pairs used in RT-qPCR

Gene	Forward primers (5′ to 3′)	Reverse primers (5′ to 3′)
COL-1	GAGGGCCAAGACGAAGACATC	CAGATCACGTCATCGCACAAC
FAK	CTCCTACTGCCAACCTGGAC	GCCGACTTCCTTCACCATAG
VCL	TCAGATGAGGTGACTCGGTTGG	TTATGGTTGGGATTCGCTCACA
ITGB1	TGTGTCAGACCTGCCTTGGTG	AGGAACATTCCTGTGTGCATGTG

GAPDH	GGACCTGACCTGCCGTCTAG	GTAGCCCAGGATGCCCTTGA

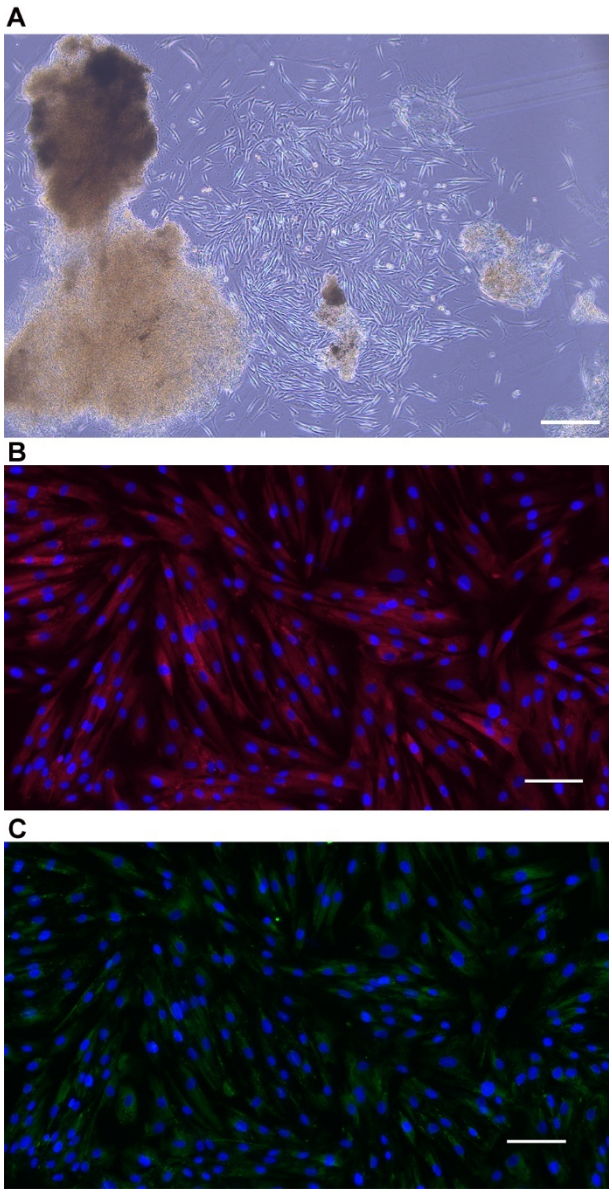

Figure 1. A. Characterisation of fibroblasts isolated from human gingival tissue. Bright-field image of human gingival fibroblasts (HGFs) migrating from tissue explants after 15 days of culture. Scale bar = 500 μm; **B, C.** Immunofluorescence staining of HGFs cultured on glass slides for two days, showing positive expression of (B) CD90 and (C) vimentin, as described in Methods section. Scale bar = 100 μm

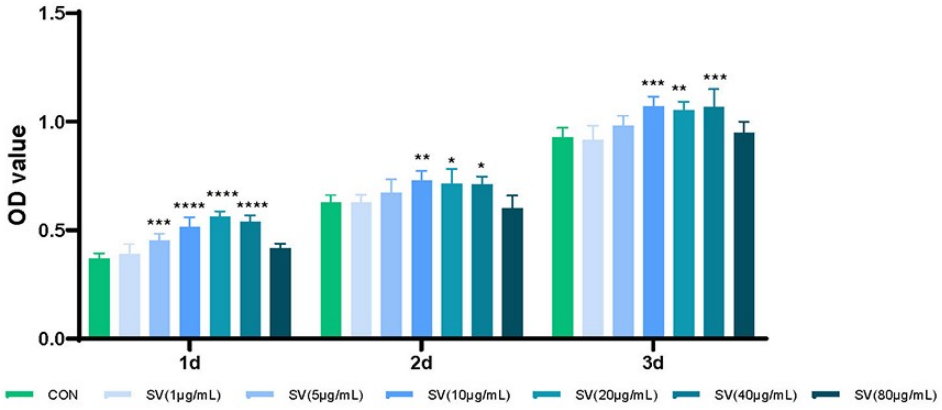

Figure 2. Effect of PEG4-SV peptide on human gingival fibroblasts proliferation activity after one, two and three days of culture. Data presented as mean ± standard deviation
$^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$, (n = 3) compared to control (CON) group
OD — optical density

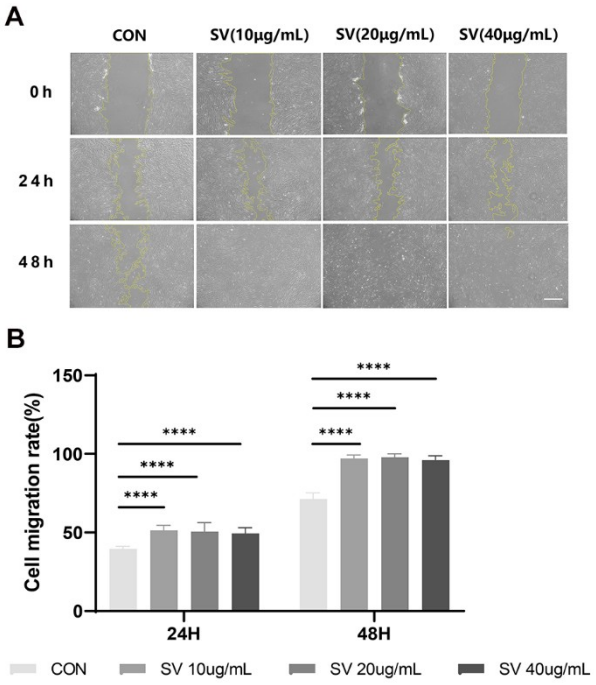

Figure 3. Effect of PEG4-SV on migration of human gingival fibroblasts was assessed using an *in vitro* wound healing assay (**A**) (scale bar = 100 μm) and analysed by cell migration rate

(**B**) after 0, 24 and 48 hours. Data presented as mean ± SD

$^{****}P \leq 0.0001$, (n = 3)

CON — control group

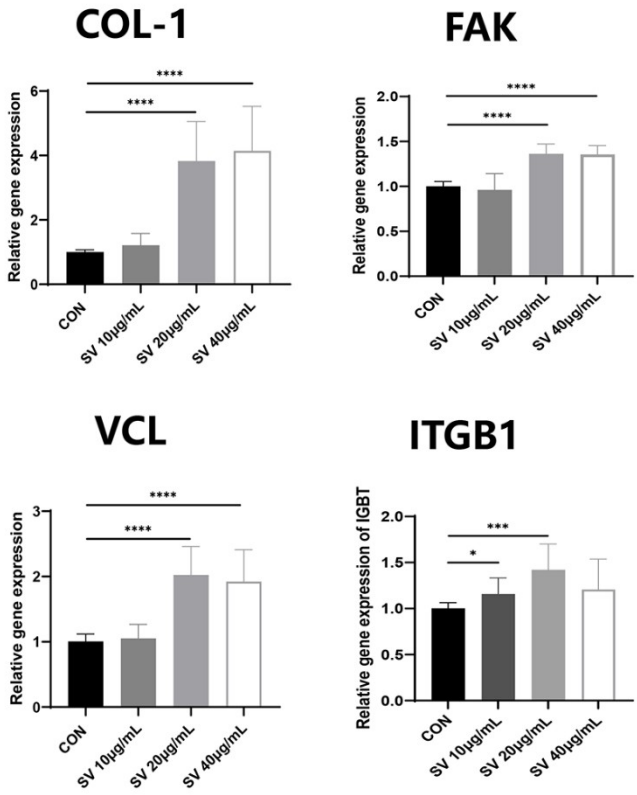

Figure 4. Effect of PEG4-SV on expression of human gingival fibroblasts adhesion-related genes COL-1, FAK, VCL and ITGB1. Genes mRNA levels were measured by RT-qPCR after 72 h of culture as described in Methods. Data presented as mean ± standard deviation $^{*}P \leq 0.05$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$, (n = 3)

COL-1 — collagen type I; CON — control group; FAK — focal adhesion kinase; ITGB1 — integrin β1; VCL — vinculin

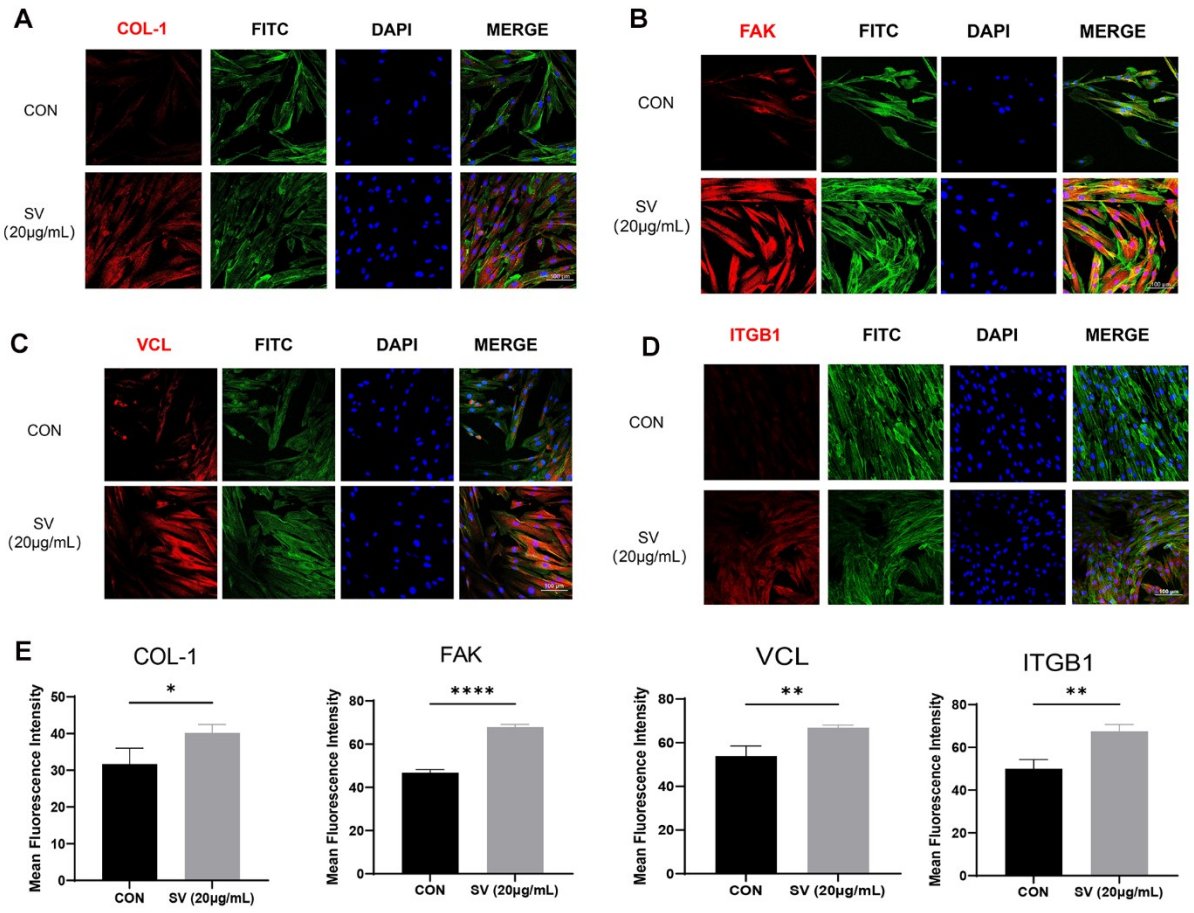

Figure 5. Effect of PEG4-SV on expression of human gingival fibroblasts adhesion proteins was analysed by immunofluorescence after 48 h of culture. **A–D** express COL-I, FAK, VCL and ITGB1, respectively. Key to colours: adhesion-related protein (red); F-actin (green); nuclei (blue); scale bar = 100 μm. Quantitative statistics of COL-I, FAK, VCL and ITGB1 expression was analysed by mean fluorescent intensity of red staining. Data presented as mean ± SD

$^{*}P < 0.05$ $^{**}P < 0.01$ $^{***}P < 0.001$ $^{****}P < 0.0001$, (n = 3)

COL-1 — collagen type I; CON — control group; DAPI — 4',6-diamidino-2-phenylindole; FAK — focal adhesion kinase; FITC — fluorescein isothiocyanate; ITGB1 — integrin β1; VCL — vinculin

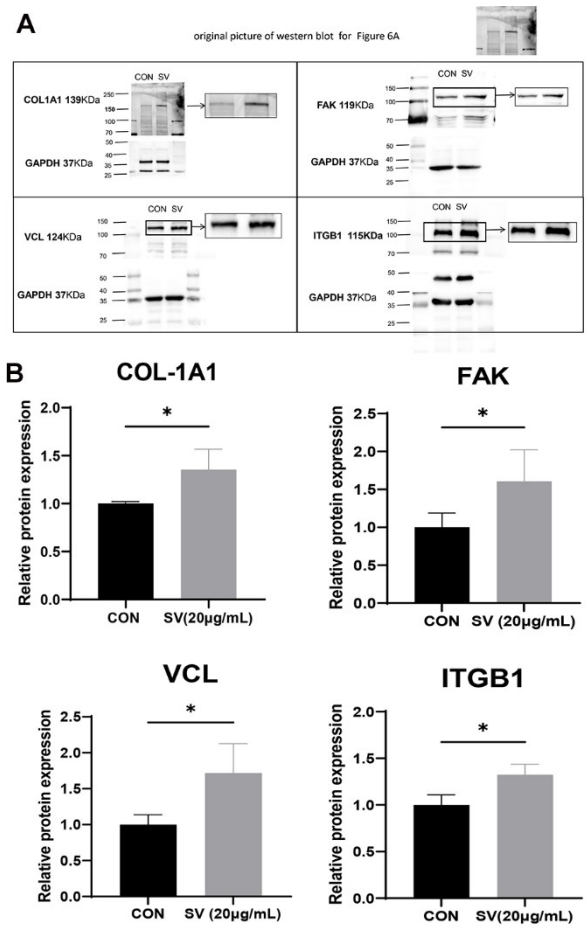

Figure 6. Effect of PEG4-SV on expression of adhesion proteins COL-1A1, FAK, VCL and ITGB1 was measured by Western blotting after 72 h of culture. Data presented as mean ± standard deviation

*P □ 0.05, (n = 3)

COL-1 — collagen type I; CON — control group; FAK — focal adhesion kinase; ITGB1 — integrin β1; VCL — vinculin