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# Integrin binding peptide promotes in vitro wound closure in the L929 mouse fibroblasts

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## ABSTRACT

***Objective.*** Molecular basis of wound healing process needs to further examined to determine the effective individual biological cues. The objective of this study was to investigate the wound closure, proliferation, and viability of L929 fibroblast when cultured with different concentration of soluble RGD peptid. ***Methods***. RGD peptide was synthesized manually on solid phase. The percentage of healed wound area for control, 0.5 mM, 1 mM, and 2 mM at each time points were analyzed by ImageJ. Cell proliferation and viability were assessed with MTT and live/ dead analysis, respectively. ***Results.*** The results of wound closure area showed that increased RGD peptide concentration in the culture improved cellular migration which enables significantly accelerated wound closure. However, RGD peptide did not dramatically augmented cell proliferation. In addition, cell viability results indicated that dead cell numbers did not critically influence by increasing the RGD peptide concentration in the culture. ***Conclusions.*** The present study showed that soluble integrin binding peptide accelerated the migration and wound closure rate of L929 fibroblasts. Delivery of soluble integrin binding peptides into the wound area may be considered as an alternative wound treatment technique in the near future after proofing the concept study with animal and clinical studies.

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***Keywords:*** Integrin binding peptide, *in vitro* scratch assay, cell migration, serum free media

## Introduction

Wound healing is a dynamic and complex process involving of synchronized actions including inflammation, regeneration, migration, proliferation, and remodeling [1, 2]. Wound healing process starts right after the skin injury and involves extensive cell-to-cell and cell extracellular matrix (ECM) communications, enhanced cell migration and action of soluble mediator [3-5]. Since wounds remain one of the major clinical problem with high morbidity and mortality rate, many different studies have been carried out to understand the molecular basis of acute and chronic wound healing processes [6, 7]. One of the critical time frame in wound healing is the proliferation and migration of fibroblasts and myofibroblasts in the surrounding tissue,which are stimulated especially for the first 3 days after the

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injury [8, 9]. The major mediators that accelerate fibroblasts proliferation and migration during wound healing requires to be determined to better understand the molecular mechanism of wound healing. The standard procedure for *in vitro* culture is based on supplementing cell culture media with fetal bovine serum (FBS). FBS is rich in growth factors and ECM such as laminin, fibronectin that enhance cell attachment, proliferation and differentiation. To comprehend individual efficacy of different proteins or bioactive peptides, it would be desirable to culture cells under serum-free media with defined biological cues. It was previously reported that serum free culture media has been developed to prevent the need for presence of bovine serum [10-12]. Kim *et al.* [13] developed serum-free culture conditions by using ECM proteins mimetic peptides to investigate the effect of different biological cues on adhesion and proliferation of chorion derived mesenchymal stem cells (MSCs). In another study, Nyegaard *et al.* [14] was initiated that 5 ng/ml epidermal growth factor (EGF) and 5 µg/ml osteopontin (OPN) in culture media induced migration of human small intestine cells. Current studies also focus on to identify peptide structures which are the small functional units of proteins to design non-immunogenic therapeutic molecules. These peptide structures have key function on cell-to-cell and cell-ECM interaction which are mediated by integrin receptors which are composed of 18α and 8β subunits and placed on cell membrane [15, 16]. The RGD (R: arginine; G: glycine; D: aspartic acid) sequence is the smallest integrin binding unit that presents in most of the ECM proteins including fibronectin, collagen, and laminin [17, 18]. RGD peptide sequence is the primary sequence to effectively modify biomaterials surface to enhance cellular adhesion and migration on synthetic surfaces [15, 16]. It was previously reported that integrin mediated cell spreading activates cell proliferation of surface dependent cells [19, 20]. Immobilized RGD peptide on substrate promotes cellular adhesion, whereas soluble form of RGD peptides inhibits the attachment of the cells and lead apoptosis by covering all the available integrin binding units of the cells [21]. For instance, Patrulea *et al.* [22] reported that RGD peptide functionalization significantly increased fibroblast adhesion and proliferation on chitosan based wound dressing. On the contrary, it has been demonstrated that soluble RGD peptide inhibited the adhesion of non-transformed human lung fibroblasts and trigger apoptosis. It was also previously described that blocking the integrin receptors with soluble RGD peptide may inhibit cell migration. Therefore, soluble RGD peptide was also introduced as possible therapeutics that showed anti-metastatic activity [23, 24]. On the contrary, Reynolds *et al.* [25] demonstrated that soluble RGD peptide can also promote the migration of the tumor cells. Furthermore, Shabbir *et al.* [26] investigated the influence of soluble RGD peptide on cell migration. They seeded HT1080 epithelial cells on RGD peptide immobilized gold substrate and explored the migration of these cells in different RGD peptide containing media. It was reported that cell migration was promoted by the presence of soluble RGD peptides because of the dynamic detachment of the integrin receptors with the substrate [26]. Although couple of studies mentioned the effect of soluble RGD peptide on different cell lines migration capacity, L929 mouse fibroblasts migration and proliferation response in *in vitro* scratch assay when cultured with different concentration of soluble peptide has yet to be studied. Based on these findings, we hypothesized that soluble RGD peptide could enhance the wound closure capacity of mouse L929 fibroblasts which are one of the model cell line of skin tissue by accelerating the migration of the cells. Hence, the objective of this study was to investigate the scratch closure rate of L929 fibroblast when cultured with different concentration of soluble RGD peptide enriched FBS free media. As it was schematically presented in Figure 1, wound closure was observed by analyzing captured micrographs at 24, 48, and 72h. The cell proliferation analysis at 24, 48, and 72 hours were assessed by cell proliferation assay. Live and dead cell analysis was also evaluated to determine cell viability at each time points.

## Methods

*Peptide Synthesis*

All the chemicals used for peptide synthesis were purchased from AAPPTEC (Louisville, KY, USA). GRGDS peptide sequence was synthesized manually on 4-Methylbenzhydrylamine (MBHA) resin (substitution = 0.67 mole/g) according the previously described procedure by Karaman *et al.* [27]. Briefly, 100 mg resin was swelledin 2ml Dimethylformamide (DMF) solution for 20 minutes. Fmoc-protecting group was removed using de-protection solution which is 20% piperidine in DMF for 20 minutes. 2 equivalents (based on resin substitution) of Fmoc-

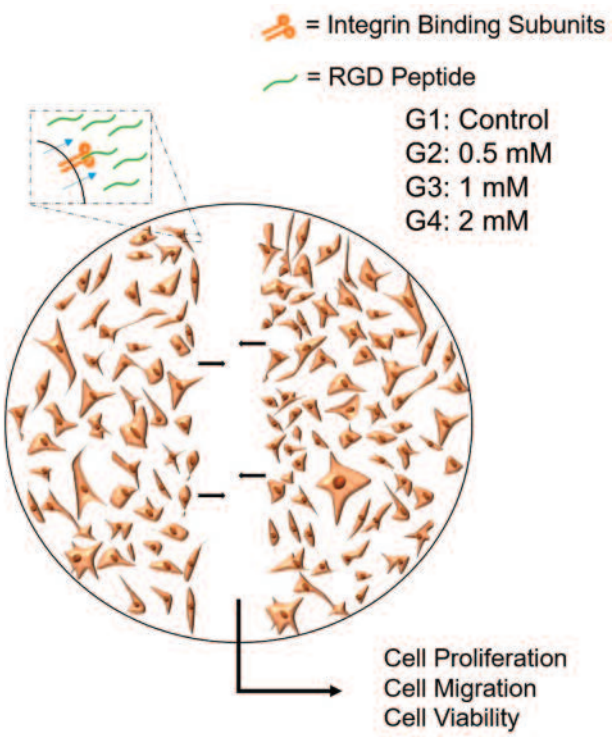


**Figure 1.** Schematic of the study. Integrin binding (RGD) peptide influence on cell proliferation, migration and viability.

protected amino acid was dissolved in DMF and added to the resin. 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexauorophosphate) (2 equiv, HBTU), hydroxybenzotriazole (2 equiv, HOBt) and N, N-diisopropylethylamine (4 equiv, DIEA) were added to mixture. Tubes were mixed in orbital shaker to proceed coupling reaction for 3 hours. Each coupling and de-protection reaction was confirmedby ninhydrin test. If the result was positive, the resin was washed with DMF and the coupling reaction was repeated until a negative result was obtained. If the test result was negative, the resin was washed with DMF, reacted with 20% piperidine in DMF for 15 min, and again washed with DMF. All amino acids were coupled using the same method. When the last amino acid was coupled, peptide was cleaved from resin by using trifluoroacetic acid (TFA): triisopropylsilane (TIPS) : DI Water solution at ratio of 95 : 2.5 : 2.5. TFA was evaporated with the rotary evaporator. The peptide was precipitated in ice-cold diethyl ether. Next, precipitated peptide was washed by ice-cold diethyl ether for three times. Finally, the resulting pellet was freeze-dried.

*Cell Culture*

Mouse fibroblasts (L929) were obtained from Ege University Research Group of Animal Cell Culture and Tissue Engineering Laboratory and cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma Aldrich, St. Louis, Missouri, USA), supplemented with 10% FBS (Sigma Aldrich, St. Louis, Missouri, USA), 1% L-glutamin (Gibco,Grand Island,USA) and 0.1% penicillin/streptomycin in a humidified incubator containing 5% CO□ and 95% air at 37ºC. Cells were kept in exponential phase and for this study cells at passage three were used.

*Wound Scratch Test Assay*

$5\times10^4$ cells/cm$^2$ L929 cells were seeded on 24-well plate and incubated at 37ºC for 24 hours in the 5% of CO2 atmosphere. A linear scratch was created in the confluent monolayer by gently scraping with sterile 200 µl pipette tips. Afterward, the cellular debris was removed by rinsing these cells using sterile PBS. RGD peptide was dissolved in sterilized water, sonicated, sterilized through filtering, and stored at −20ºC. Fresh medium (with no FBS) containing RGD at concentrations of 0.5 mM, 1 mM and 2mM was added to the media. Serum free media with no RGD peptide was used as a control. Promotion of wound closure observed by capturing micrographs with florescence attached phase-contrast microscope (CKX41, Olympus, Hamburg, Germany) at 0h, 24h, 48h and 72h, respectively. The percentage of healed wound area for control, 0.5 mM, 1 mM, and 2mM at each time points were analyzed by using the ImageJ software (NIH, Bethesda, MD, http://www.rsb.info.nih.gov/ij).

*Cell Proliferation and Viability Analysis*

Cell proliferation analysis was assessed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Vybrant® MTT Cell Proliferation Assay Kit, Invitrogen, Waltham, MA, USA) assay according to the manufacturer's instructions, after 1, 3, and 5 days of incubation [28]. Briefly, MTT solution (5 mg/mL) was added into culture medium (with 10% concentration) and incubated for 2 h at 37°C. Next, the medium was replaced with 500 µL DMSO (Sigma Aldrich, St. Louis, MO, USA), and the optical density for each well was measured at 540 nm using a Synergy™ HTX Multi-Mode Microplate Reader (BioTek, Winooski, VT, USA). Measured absorbance values were correlated to the equivalent number of cells by using a calibration curve constructed with reference number of cells. The effect of RGD peptide addition in serum-free media on cell proliferation were determined. After 72 hours of culture, live/ dead double fluorescence staining kit (Dojindo EU,

Munich, Germany) was used to evaluate viability of L929 fibroblasts on well plates by fluorescence microscopy. Briefly, the viable cells (Calcein-AM, green fluorescence) and dead cells (propidium iodide, red fluorescence) were studied using a fluorescence microscope after 15 min of incubation in a culture medium.

*Statistical Analysis*

Three independent experiments were performed and in each experiment, three cell seeded wells were used for each experimental groups. The images taken from same cell seeded wells for each group at different time points. All data were expressed as mean ± standard error and were statistically analyzed by one-way ANOVA (SPSS 12.0, SPSS GmbH, Germany) and the Student-Newman-Keuls method as a post hoc test. Significant differences between groups were determined at *p* values at least less than 0.05.

## Results

*Wound Closure Measurement*

After L929 fibroblasts reached confluency level, scratch was occurred in order to assess wound healing capacity of cell when cultured with different concentrations of RGD peptide. The micrographs of control, 0.5 mM, 1 mM, and 2 mM RGD at 0, 24, 48, and 72 h were shown in Figure 2. The percentage of healed wound area for control, 0.5 mM, 1 mM, and 2 mM RGD groups at 0, 24, 48, and 72 h was assessed by ImageJ and presented in Figure 3. By 24 h, it is evident that the scratch closure occurred at a faster rate in the presence of different concentration of RGD peptides. For instance, wound closure area significantly increased in 2 mM RGD (29.30 ± 0.9%), 1 mM RGD (28.00 ± 1.1%), and 0.5 mM RGD (22.82 ± 4.03%) compared to control (21.81 ± 0.56%). Similarly, at 48 h, significantly faster wound closure in RGD groups was observed in 2 mM RGD (45.47 ± 1.47%) followed by 1 mM RGD (35.89 ± 1.3%) and 0.5 mM RGD (34.34 ± 0.55 %) compared to control. Additionally, after 72h, 2mM RGD (58,67 ± 1.49%) was the highest in terms of wound closure are among

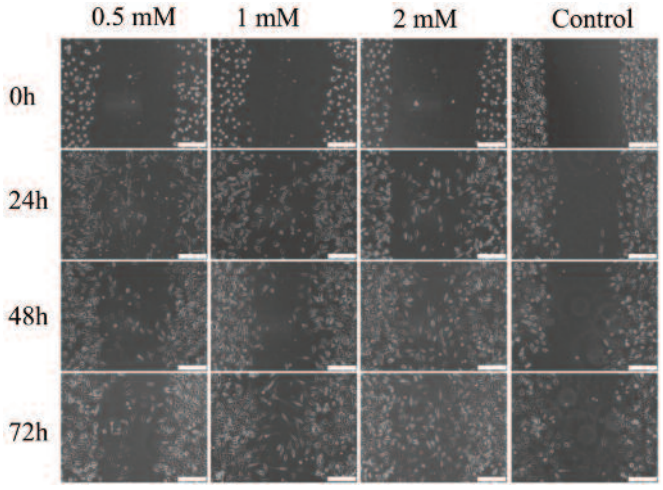


**Figure 2.**Micrographs of in vitro wound closure assay of control, 0.5 mM, 1 mM, 2 mM RGD peptide groups at 0, 24, 48, and 72 hours. Scale bar is equal to 200 µm.

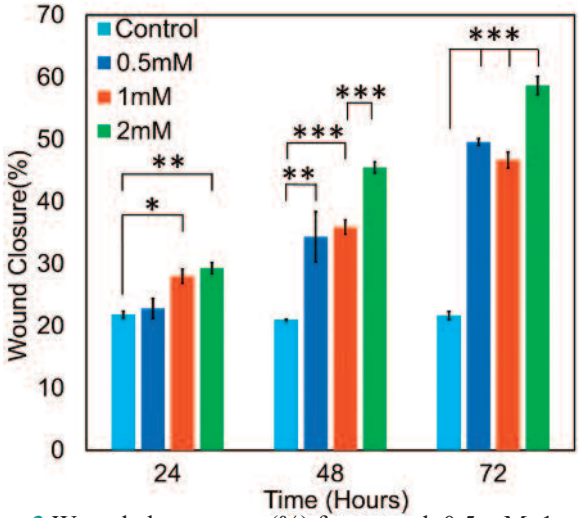


**Figure 3.**Wound closure area (%) for control, 0.5 mM, 1 mM, and 2 mM RGD peptide groups at 24, 48 and 72 hours by using Image J. Error bars represent mean ± SE (n = 5) (significant differences were determined by one-way ANOVA (Newman-Keuls multiple comparison test, (*p < 0.05, **p < 0.01,***p < 0.001).

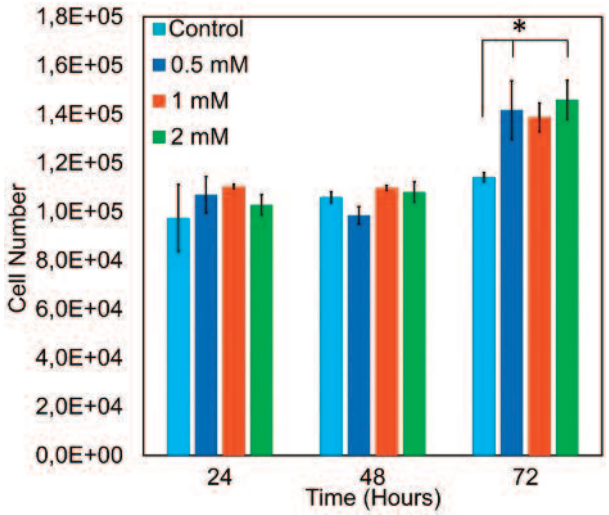


**Figure 4.**Cell numbers of control, 0.5 mM, 1 mM, and 2 mM RGD peptide groups at 24, 48, and 72 hours. Error bars represent mean ± SE (n = 5) (significant differences were determined by one-way ANOVA (Newman-Keuls multiple comparison test, (*p < 0.05, **p < 0.01,□***p < 0.001).

RGD groups, followed by 0.5 mM RGD (49.57 ± 1.3%) and 1mM RGD (46.69 ± 0.27%). The results of wound closure area showed that RGD treatment improved cellular migration which enables significantly accelerated wound closure.

*Cell Proliferation and Viability during Wound Closure*

The effect of RGD peptide concentrations of 0.5 mM, 1 mM, and 2 mM on cell proliferation at 24, 48, and 72 h were presented in Figure 4. The results showed that RGD peptide implementation did not affect the cell proliferation at early time points of culture including 24 and 48 h. However, at 72 h, cell number in 2 mM RGD (145,885 ± 8,037), 1 mM (138,663 ± 5,931), and 0.5 mM RGD (141,672.62 ± 12,028) was significantly higher compared to control group (114,114 ± 1,970). The results of MTT assay suggested that increased RGD peptide enriched culture media improved cellular adhesion and proliferation in the late phase of wound healing. Fluorescent microscopy images of live/ dead staining analysis in control, 0.5 mM, 1 mM, and 2 mM at 72 h were shown in Figure 5a, b, c, and d, respectively. The results indicated that dead cell numbers did not critically differ by increasing the RGD peptide concentration in the culture.

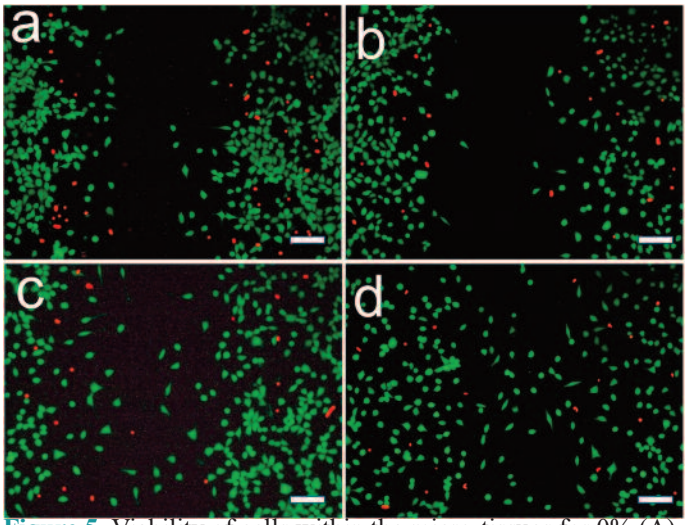


**Figure 5.** Viability of cells within the micro-tissues for 0% (A), 1% (B), 5% (C), and 10% (D) FBS groups was examined on day 7 (green: LIVE cells, red: DEAD cells) (20x). Scale bar represents 100 µm size.

## Discussion

In this study, to precisely observe the effects of three different concentrations of RGD peptides on *in vitro* wound closure, proliferation, and viability of L929 fibroblasts, experiments were conducted with serum-free media. As mentioned earlier, soluble RGD peptides have converse effect on cell behaviors. It was indicated that soluble RGD peptide, when treated with adherent cells prior to cell seeding, prevented the adhesion of cells [21, 23]. The potential reason for this function could be blockage of all integrin binding points of these cells with soluble RGD peptide; therefore, cell adhesion was inhibited. On the other hand, it was also reported that due to the dynamic detachment of the integrin receptor in the presence of soluble RGD peptide, migration of tumor cells was augmented [25].

Our results clearly indicated that increasing the RGD peptide concentration in the culture media significantly accelerated the wound closure of L929 fibroblast (Figure 2 and Figure 3). It could be directly related with the enhanced migration and proliferation capacity of the cells in the presence of RGD peptide. It was also observed that even with higher concentration of soluble RGD peptide did not critically affect cell proliferation specifically at the early time points of *in vitro* wound healing (Figure 4). However, wound closure area was significantly increased by increasing the soluble RGD peptide concentration at each time point. The reason for such rate on cell proliferation might be due to the culture of cells without serum concentration. Although different concentration of RGD peptide was supplemented into the test groups, still there was no serum in the culture therefore no early proliferation rate was observed. From 48 to 72 hours, L929 fibroblasts might start using soluble RGD peptide as a source of amino acids and it might be one of the reason that partially accelerate the proliferation of L929 fibroblasts. Our data showed that although different RGD peptide concentration did not practically affect the proliferation rate, it was clearly seen that higher RGD peptide concentration significantly trigger the migration rate of L929 fibroblasts. Based on these findings, we hypothesize that soluble RGD peptides significantly influence the migration of L929 fibroblasts. The reason of enhanced cell migration caused by soluble RGD peptide may be related with accelerated attachment of the integrin receptors. Similarly, Jones *et al.* [29] reported that addition of RGD sequence including insulin-like growth factor binding protein (IGFBP-1) in the cell culture medium significantly enhanced the migration of CHO cells.

Another possible explanation of such trend on enhanced cell migration by increasing RGD peptide

concentration might the activation ofsignalingpathways such as, focal adhesion,caspase-3,FAK proteolysis [21, 26, 30]. In a previous study, it has been compared the rates of cell migration and viability in the presence of soluble peptide in concentrations. It was reported that the addition of soluble peptide increased the rate of disconnection of focal adhesions at the back of the cell, which may induce higher migration levels and the presence of a soluble peptide helps to block individual communications from reassembling and allows an "unzipping" of the polyvalent complex [26].

Cell viability results revealed that increasing the soluble RGD peptide concentration did not critically affect cell viability. Although it was previously reported that RGD-containing peptides provoked apoptosis, we did not observed similar results [31, 32]. Buckley *et al.* [21] reported that RGD peptides induced apoptosis through direct caspase-3 activation if adherent cells detach from their substrate. In that case, soluble RGD peptides prevented the attachment of the cells by blocking all the available integrin binding units of the cells and lead apoptosis. However, in these study as predicted from live/ dead staining micrographs, soluble RGD peptide did not inhibit cell viability. The potential reason of not leading major cell death might be addition of RGD peptide to already attached fibroblasts culture. It is also recommended that these claim needs to further investigate with apoptosis assay.

## Conclusions

In the present study, we investigated the effect of various concentration of soluble RGD peptide on wound closure capacity, proliferation, and viability of L929 fibroblasts *in vitro*. By incorporating soluble RGD with different concentrations including 0.5 mM, 1 mM, and 2 mM to cell culture media, L929 fibroblasts exhibited greater cell proliferation and wound closure capacity compared to control group.Taken together, soluble RGD peptide applications may be an alternative wound treatment technique in the near future after proofing the concept study with animal and clinical studies. Experiments to understand the efficacy of soluble RGD peptide application on an in vivo wound model are underway.

*Conflict of interest*

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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## References

[1] Labler L, Mica L, Härter L, Trentz O, Keel M. [Influence of VAC-therapy on cytokines and growth factors in traumatic wounds]. Zentralbl Chir 2006;131:S62-7. [Article in German]
[2] Rivera AE, Spencer JM. Clinical aspects of full-thickness wound healing. Clin Dermatol 2007;25:39-48.
[3] Robson MC, Steed DL, Franz MG. Wound healing: biologic features and approaches to maximize healing trajectories. Cur Probl Surg 2001;38:72-140.
[4] Natarajan S, Williamson D, Stiltz AJ, Harding K. Advances in wound care and healing technology. Am J Clin Dermatol 2000;1:269-75.
[5] Strong AL, Neumeister MW, Levi B. Stem cells and tissue engineering: regeneration of the skin and its contents. Clin Plast Surg 2017;44:635-650.
[6] Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res 2009;37:1528-42.
[7] Gonzalez AC, Costa TF, Andrade ZA, Medrado AR. Wound healing - A literature review. An Bras Dermatol 2016;91:614-20.
[8] Witte MB, Barbul A. General principles of wound healing. Surg Clin North Am. 1997;77:509-28.
[9] Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. Sci Transl Med 2014;6:265sr6.
[10] Boyce ST, Ham RG. Calcium-regulated differentiation of normal human epidermal keratinocytes in chemically defined clonal culture and serum-free serial culture. J Invest Dermatol 1983;81:33s-40s.
[11] Wang HJ, Chen TM, Cheng LF, Cheng TY, Tung YM. Human keratinocyte culture using porcine pituitary extract in serum-free medium. Burns 1995;21:503-6.
[12] Higham MC, Dawson R, Szabo M, Short R, Haddow DB, MacNeil S. Development of a stable chemically defined surface for the culture of human keratinocytes under serum-free conditions for clinical use. Tissue Eng 2003;9:919-30.
[13] Kim JH, Jekarl DW, Kim M, Oh EJ, Kim Y, Park IY, et al. Effects of ECM protein mimetics on adhesion and proliferation of chorion derived mesenchymal stem cells. Int J Med Sci 2014;11:298-308.
[14] Nyegaard S, Christensen B, Rasmussen JT. An optimized method for accurate quantification of cell migration using human small intestine cells. Metab Eng Commun 2016;3:76-83.
[15] Hersel U, Dahmen C, Kessler H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. Biomaterials 2003;24:4385-415.
[16] Heath DE, Cooper SL. The development of polymeric biomaterials inspired by the extracellular matrix. J Biomater Sci Polym Ed 2017;28:1051-69.
[17] Pierschbacher MD, Ruoslahti E. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature 1984;309:30-3.
[18] Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. Science 1987;238:491-7.
[19] Giancotti FG. Complexity and specificity of integrin signalling. Nat Cell Biol 2000;2:E13-4.
[20] Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. Science 1997;276:1425-8.
[21] Buckley CD, Pilling D, Henriquez NV, Parsonage G, Threlfall K,

Scheel-Toellner D, et al. RGD peptides induce apoptosis by direct caspase-3 activation. Nature 1999;397:534-9.
[22] Patrulea V, Hirt-Burri N, Jeannerat A, Applegate L, Ostafe V, Jordan O, et al. Peptide-decorated chitosan derivatives enhance fibroblast adhesion and proliferation in wound healing. Carbohydr Polym 2016;142:114-23.
[23] Brenner W, Gross S, Steinbach F, Horn S, Hohenfellner R, Thüroff JW. Differential inhibition of renal cancer cell invasion mediated by fibronectin, collagen IV and laminin. Cancer Lett 2000;155:199-205.
[24] Tolomelli A, Galletti P, Baiula M, Giacomini D. Can integrin agonists have cards to play against cancer? A literature survey of small molecules integrin activators. Cancers (Basel) 2017;9:E78.
[25] Reynolds AR, Hart IR, Watson AR, Welti JC, Silva RG, Robinson SD, et al. Stimulation of tumor growth and angiogenesis by low concentrations of RGD-mimetic integrin inhibitors. Nat Med 2009;15:392-400.
[26] Shabbir SH, Eisenberg JL, Mrksich M. An inhibitor of a cell adhesion receptor stimulates cell migration. Angew Chem Int Ed Eng 2010;49:7706-9.
[27] Karaman O, Kumar A, Moeinzadeh S, He X, Cui T, Jabbari E. Effect of surface modification of nanofibres with glutamic acid peptide on calcium phosphate nucleation and osteogenic differentiation of marrow stromal cells. J Tissue Eng Regen Med 2016;10:E132-46.
[28] Liu Y, Peterson DA, Kimura H, Schubert D. Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. J Neurochem 1997;69:581-93.
[29] Jones JI, Gockerman A, Busby WH, Wright G, Clemmons DR. Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the alpha 5 beta 1 integrin by means of its Arg-Gly-Asp sequence. Proc Natl Acad Sci 1993;90:10553-7.
[30] Hadden HL, Henke CA. Induction of lung fibroblast apoptosis by soluble fibronectin peptides. Am J Respir Crit Care Med 2000;162:1553-60.
[31] Frisch SM, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. J Cell Biol 1994;124:619-26.
[32] Hayman EG, Pierschbacher MD, Ruoslahti E. Detachment of cells from culture substrate by soluble fibronectin peptides. J Cell Biol 1985;100:1948-54.