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RESEARCH ARTICLE

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Therapeutic effects of a recombinant human collagen peptide bioscaffold with human adipose-derived stem cells on impaired wound healing after radiotherapy

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Abstract

Chronic changes following radiotherapy include alterations in tissue-resident stem cells and vasculatures, which can lead to impaired wound healing. In this study, novel recombinant human collagen peptide (rhCP) scaffolds were evaluated as a biomaterial carrier for cellular regenerative therapy. Human adipose-derived stem cells (hASCs) were successfully cultured on rhCP scaffolds. By hASC culture on rhCP, microarray assay indicated that expression of genes related to cell proliferation and extracellular matrix production was upregulated. Pathway analyses revealed that signaling pathways related to inflammatory suppression and cell growth promotion were activated as well as signaling pathways consistent with some growth factors including vascular endothelial growth factor, hepatocyte growth factor, and transforming growth factor beta, although gene expression of these growth factors was not upregulated. These findings suggest the rhCP scaffold showed similar biological actions to cytokines regulating cell growth and immunity. In subsequent impaired wound healing experiments using a locally irradiated (20 Gray) mouse, wound treatment with rhCP sponges combined with cultured hASCs and human umbilical vein endothelial cells accelerated wound closure compared with wounds treated with rhCP with hASCs alone, rhCP only, and control (dressing alone), with better healing observed according to this order. These results indicating the therapeutic value of rhCP scaffolds as a topical biomaterial dressing and a biocarrier of stem cells and vascular endothelial cells for regenerating therapies. The combination of rhCP and functional cells was suggested to be a potential tool for revitalizing stem cell-depleted conditions such as radiation tissue damage.

KEYWORDS

adipose-derived stem cells, cell therapy, endothelial cells, irradiation, wound healing

1 | INTRODUCTION

Localized radiotherapy is a commonly-used, effective treatment for various types of cancers; however, it frequently causes acute and chronic tissue injury. Although acute radiation injuries, such as erythema or tissue erosions, heal over relatively short time periods (usually within weeks; Koenig, Wolff, Mettler, & Wagner, 2001), chronic radiation injury alters the tissue microenvironment, including ischemia, fibrosis, and depleting sensitive stem cell niches (Coppes, van der Goot, & Lombaert, 2009; Harfouche & Martin, 2010). These chronic tissue alterations can lead to impaired wound healing and make it difficult to treat with surgical interventions (Kim, Kolozsvary, Jenrow, & Brown, 2013; Zawaski et al., 2014). Innovative treatments are needed to improve outcomes for patients experiencing long-term tissue complications following radiotherapy.

Recently, autologous fat grafting has been used in the field of plastic surgery to ameliorate radiation-induced tissue changes (Akita, Yoshimoto, Ohtsuru, Hirano, & Yamashita, 2012; Haubner, Ohmann, Pohl, Strutz, & Gassner, 2012; Luan et al., 2016; Mizuno & Hyakusoku, 2010; Rigotti et al., 2007). The clinical benefits of these interventions

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have been attributed to the regenerative properties of fat tissue cells: undifferentiated, multipotent adipose-derived stem cells (ASCs), which produce angiogenic and antiapoptotic growth factors and modulate inflammation and immunity (Butala et al., 2012; Hong, Traktuev, & March, 2010; Kuo et al., 2011; Mashiko & Yoshimura, 2015; Phinney & Prockop, 2007), and vascular endothelial cells (ECs), which serve as paracrine mediators and upregulators of ASC functionality (Lin et al., 2014; Mashiko et al., 2017). Stem cell-replacement therapy for stem cell-depleted conditions, specifically including postirradiation tissue injury, seems to be a rational solution, and some reports have already demonstrated the therapeutic potential of human ASCs (hASCs) for irradiated tissue (Bajada, Mazakova, Richardson, & Ashammakhi, 2008; Chang, Qu, Wang, & Dong, 2015; Hasdemir et al., 2015; Li et al., 2015). Optimizing regenerative cell therapies for stem cell-depleted tissues, however, still requires the determination of which cell types, preparation methods, and administration techniques are most effective.

Recombinant human collagen peptide (rhCP) has been developed as a biomaterial scaffold for three-dimensional cell culture. Although most of current naturally based biomaterials (e.g., collagen and gelatin) are prepared from mammalian tissues (Dunn, 2012; Kimura, Inamoto, & Tabata, 2010; Mano et al., 2007), rhCP used in this study is a recently developed peptide-based product containing multiple Arg-Gly-Asp (RGD) motifs that are ligands for some types of integrin receptors (Pawelec et al., 2017), which is totally different from other materials of natural or synthetic collagen fibres. The rhCP is not only expected to enhance cellular activities through integrin receptors but also is producible in various forms, including porous sponge and microspheres. In this study, we precultured hASCs and ECs on rhCP sponges and applied these sponges in a mouse postradiation skin ulcer healing model in order to investigate the therapeutic potential of these cell populations, as well as the effects of combination therapies, for enhancing healing and regenerating capacity of irradiated tissue.

2 | MATERIALS AND METHODS

2.1 | Human cell preparation

Human liposuction aspirates were obtained from 15 healthy female donors aged 43.2 ± 9.8 years (mean ± *SD*) with an average body mass index of 22.6 ± 2.1 kg/m^2 who underwent liposuction of the abdomen or thighs. Each patient provided written informed consent, and the research protocol was approved by the institutional review board. The stromal vascular fraction (SVF) was isolated from the aspirated fat tissue as described previously (Yoshimura et al., 2006). Briefly, aspirated fat tissue was digested in 0.075% collagenase (Wako Pure Chemical Industries, Osaka, Japan) at 37 °C for 30 min on a shaker. After centrifugation (800 *g* for 10 min), SVF cells were obtained as cellular pellets, filtered through 100/70/40-μm meshes, and resuspended. The SVF cells were plated at a density of 5 × 10^5 nucleated cells/100-mm dish and cultured at 37 °C in an atmosphere of 5% carbon dioxide in humid air. Cells were grown in Dulbecco's Modified Eagle Medium with Ham's F-12 (DMEM/F12; Wako Pure Chemical Industries, Osaka, Japan) supplemented with 10% fetal bovine serum. Primary cells were cultured for 7 days and obtained cells were defined as hASCs at passage 0 (P0). Human umbilical vein endothelial cells (HUVECs; Lonza, Walkersville, MD) were grown in Endothelial Cell Growth Medium-2 (EGM-2; Lonza, Walkersville, MD). Cells were passaged every week by trypsinization, and hASCs and HUVECs at P1 to P3 were used in the following experiments.

2.2 | rhCP scaffold preparation

Recombinant collagen type-I peptide (Cellnest(r), Fujifilm Corporation, Tokyo, Japan) were purchased from Wako Chemicals (Tokyo, Japan). The peptides were dissolved in 1% with water-for-injection (Hikari Pharmaceutical) and lyophilized. Lyophilized porous peptide blocks were cut into 7 (diameter) × 0.6 (thickness) mm cake. The cakes were thermally cross linked under nitrogen atmosphere for 4 hr at 145 °C.

2.3 | Cell loading and culture on the rhCP scaffolds

Cell loading was performed by applying 60 μl of the hASC suspension (DMEM/F12) onto an rhCP scaffold, which was then placed in a nonadhesive 48-well plate. After incubation for 2 hr to allow cells to adhere without spreading out of the rhCP, 200 μl of DMEM/F12 was added, and the cells were then cultured at 37 °C in an atmosphere of 5% carbon dioxide in humid air (Figure 1a).

The cells and rhCP were treated with a fluorescent dye mixture of Hoechst 33342 (Dojindo, Kumamoto, Japan) and PI (Sigma-Aldrich, St. Louis, USA) for 1 min, washed twice with phosphate buffered saline (PBS) to remove excess dye molecules, and then observed using a BZ-X700 fluorescent microscope (Keyense, Osaka, Japan). Z-stack fluorescence images (200-μm, collected in 2-μm steps) were used for analysis.

2.4 | Scanning electron microscopy

The rhCP with no cells, and with seeded number of 1 × 10^5 hASCs cultured for 5 days were fixed in 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M, pH 7.2 sodium cacodylate buffer, and then postfixed in 2% osmium tetroxide with the same buffer at room temperature for 2 hr. The specimens were dehydrated by passage through ascending alcohol concentrations and isoamyl acetate, then critical point dried with liquid carbon dioxide and sputter coated with platinum-palladium. The specimens were observed with a scanning electron microscope (SEM; S-3500N, Hitachi, Tokyo, Japan) at 25 kV.

2.5 | Histological analysis

Seeded number of 1 × 10^5 hASCs cultured for 5 days on rhCP were fixed in 4% formaldehyde and then processed by paraffin block embedding, using standard techniques to prepare paraffin sections (5 μm thick). The histology was visualized by hematoxylin and eosin staining and was observed using a light microscopy (Keyense BZ-X700).

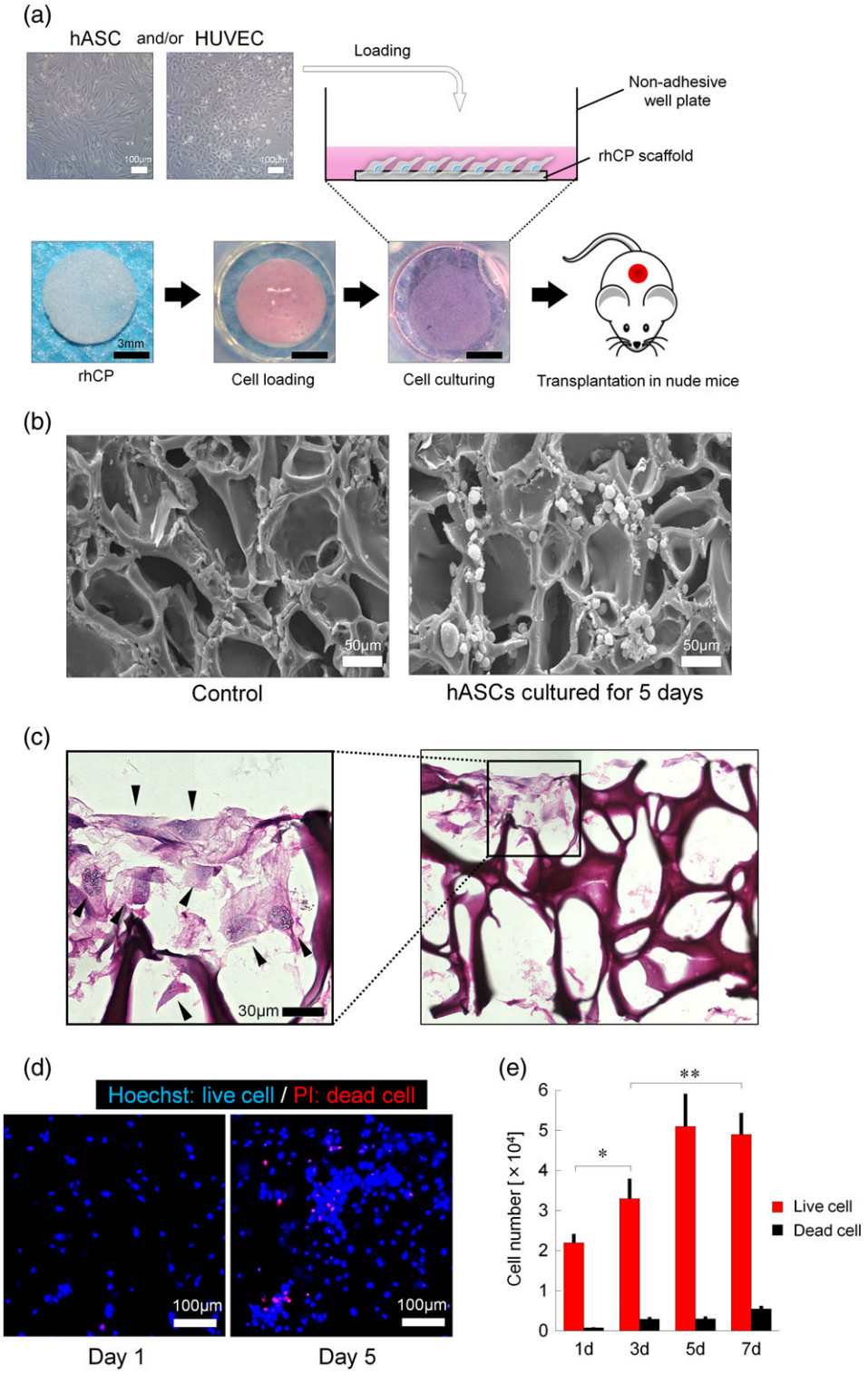

FIGURE 1 Recombinant human collagen peptide (rhCP) characteristics and cellular biocompatibility (a) schematic representation of the cell loading and transplantation methods, with microscopic views of cultured human adipose-derived stem cells (hASCs) and human umbilical vein endothelial cells (HUVECs; scale bars = 100 μm) and macroscopic views of rhCP (scale bars = 3 mm). (b) Scanning electron microscopy micrographs of rhCP with no cells (*left*) and seeded number of 1×10^5 hASCs cultured for 5 days (*right*; scale bars = 50 μm). (c) Histological view of seeded number of 1×10^5 hASCs cultured for 5 days on rhCP (hematoxylin and eosin stain). Most of the surviving cells are localized in the superficial part of the rhCP (scale bars = 30 μm). (d) Fluorescence microscopic images of hASCs after 1 and 5 days of cultivation with initial seeding number of 1×10^5. Hoechst/PI staining shows live cells as blue, and dead cells as red (scale bars = 100 μm). (e) Proliferation of hASCs loaded on rhCP after 1, 3, 5, and 7 days of culture ($n = 3$; $^*p < .05$; $^{**}p < .01$) [Colour figure can be viewed at wileyonlinelibrary.com]

2.6 | Microarray analysis of hASCs cultured with rhCP or in normal dishes

Comparative microarray analysis was performed on hASCs cultured on rhCP scaffolds and those cultured on a normal adhesive dish for 2 days. After cultivation, hASCs cultured on rhCP scaffolds were mechanically homogenized and dissolved with Isogen (Nippon Gene, Tokyo, Japan), and fine fragments of the rhCP matrix were separated and discarded using centrifugation (12,000 *g*, 4 °C, 10 min). Alternately, hASCs cultured in normal culture dishes were harvested with 0.25% trypsin and 2-mM ethylenediaminetetraacetic acid for 5 min at 37 °C, and the cell suspension was collected and dissolved with Isogen. Total RNA for each group was purified using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's directions. Fluorescent-labeled complementary RNA was synthesized using a Quick Amp Labeling Kit (Agilent Technologies, Santa Clara, CA, http://www.agilent.com). Labeled complementary RNAs were hybridized with the SurePrint G3 Human GE Microarray 8360K (G4851A; Agilent Technologies). Microarrays were scanned using a G2505C microarray scanner, and the results were analysed using Feature Extraction Software (Agilent Technologies). Finally, we analysed gene expression using GeneSpring GX Software, Version 12.5 (Agilent Technologies).

2.7 | Mice model for impaired wound healing of irradiated tissue

All animals were cared and used for in accordance with institutional guidelines and the animal committee approval. Seven-week-old, male Balb/c nude (immunocompromised) mice were obtained from Japan CLEA, Inc. (Tokyo, Japan). The irradiated mouse model was created to simulate impaired wound healing processes in the setting of chronic radiation tissue damage. Mice used in this protocol underwent the following procedures: After induction of general anaesthesia with a 2.5% isoflurane/oxygen mixture at 2 L per minute, the skin of the back was selectively irradiated using an X-ray apparatus (MediXtec Japan Corporation, Chiba, Japan) with a customized lead shield. For each subject, a radiation dose of 10 Gray (Gy) was applied twice over successive days, so that 20 Gy was administered.

Four weeks after radiation treatment, a 6 mm full-thickness circular wound was created through the panniculus carnosus tissue layer using a sterile circular biopsy punch (Kai Industries Co., Tokyo, Japan) at the same position in the irradiated area. To avoid wound contraction, we applied a circular, silicone 12-mm-diameter splint, with internal and external diameters of 9 and 15 mm, respectively (Kyowa Industries, Saitama, Japan). The splint was placed around the perimeter of each wound and secured with glue and eight simple, interrupted 6-0 nylon sutures. Each excised skin sample was then histologically examined using Mallory-Azan staining to assess postradiation tissue effects.

Following wound creation, subjects were then assigned to one of four experimental wound treatment groups; (a) Control (dressing only), (b) rhCP soaked in PBS (no cells added), (c) rhCP with hASCs, and (d) rhCP with hASCs and HUVECs. Three mice were used in each experimental group. The cell-containing rhCP had undergone the following preparations 5 days in advance of the wound creation procedures: In the rhCP with hASCs group, 60 μl of DMEM/F12 containing 1×10^5 hASCs was pipetted onto the rhCP. In the rhCP with hASCs and HUVECs group, 30 μl of EGM-2 containing 1×10^5 hASCs and 30 μl of EGM-2 containing 1×10^5 HUVECs were pipetted onto the rhCP. Cells were then cultured on the rhCP scaffolds for 5 days.

On the day of wound creation, each rhCP scaffold was placed upside down on the wound, so that the cell-rich sides were in contact with the wound surfaces. The rhCP scaffolds were found to shrink slightly from their original size when wet and thus fitted well inside the 6 mm wounds. An occlusive dressing (Perme-roll; Nitto Medical, Osaka, Japan) was used to prevent wound drying in all groups. Wounds were sequentially photographed on Days 0, 4, 7, 10, 14, and 18 using a digital camera (Canon EOS Kiss X4, Osaka, Japan), and the photographs were evaluated to measure wound area using image analysis software (Photoshop CS6; Adobe Systems, San Jose, CA).

2.8 | Statistics

Data were analysed using SPSS package 23.0 (SPSS, Inc., Chicago, IL, USA) and Kyplot 2.0 (Freeware). Based on the Kolmogorov/Smirnov test, our data were normally distributed within the donor population. Therefore, one-way analysis of variance with Bonferroni adjustment for post hoc comparisons was used to determine significant differences between the means ($p < .05$ unless otherwise stated).

3 | RESULTS

3.1 | Human ASC culture on rhCP scaffolds

SEM of the rhCP scaffolds revealed interconnected, macroporous three-dimensional structures with micropore sizes of 80–120 μm, which are suitable for cell migration and nutrient diffusion (Figure 1b).

Histologic analysis of hASCs cultured on rhCP scaffolds stained with hematoxylin and eosin suggested that most of surviving cells were localized in the superficial layers of the rhCP (Figure 1c).

To evaluate hASC adhesion and proliferation on the rhCP scaffolds, cell number and viability during the culture period were measured by Hoechst-propidium iodide (PI) double staining. Preliminary testing revealed that a seeding number of 1×10^5 cells and a culture period of 5 days achieved good hASC attachment, proliferation, and viability within the rhCP scaffold (Figure 1d,e), and these standard practices were then used for subsequent experiments.

3.2 | Comparative gene expression of hASCs cultured on rhCPs or normal dishes

Microarray analyses were performed to analyse differences in gene expression between hASCs cultured on an rhCP scaffold and hASCs cultured in a normal dish. Gene ontology analyses between the two populations indicated several characteristic ontologies, as summarized in Figure 2a. Among these, cell cycle genes and wound healing genes (in particular, those related to the extracellular matrix [ECM]) were upregulated in the rhCP group, as visualized by heat mapping (Figure 2b). Pathway analyses revealed that hASCs cultured on rhCP had gene expression patterns consistent with inflammatory suppression and cell growth promotion. In addition, downstream genes of

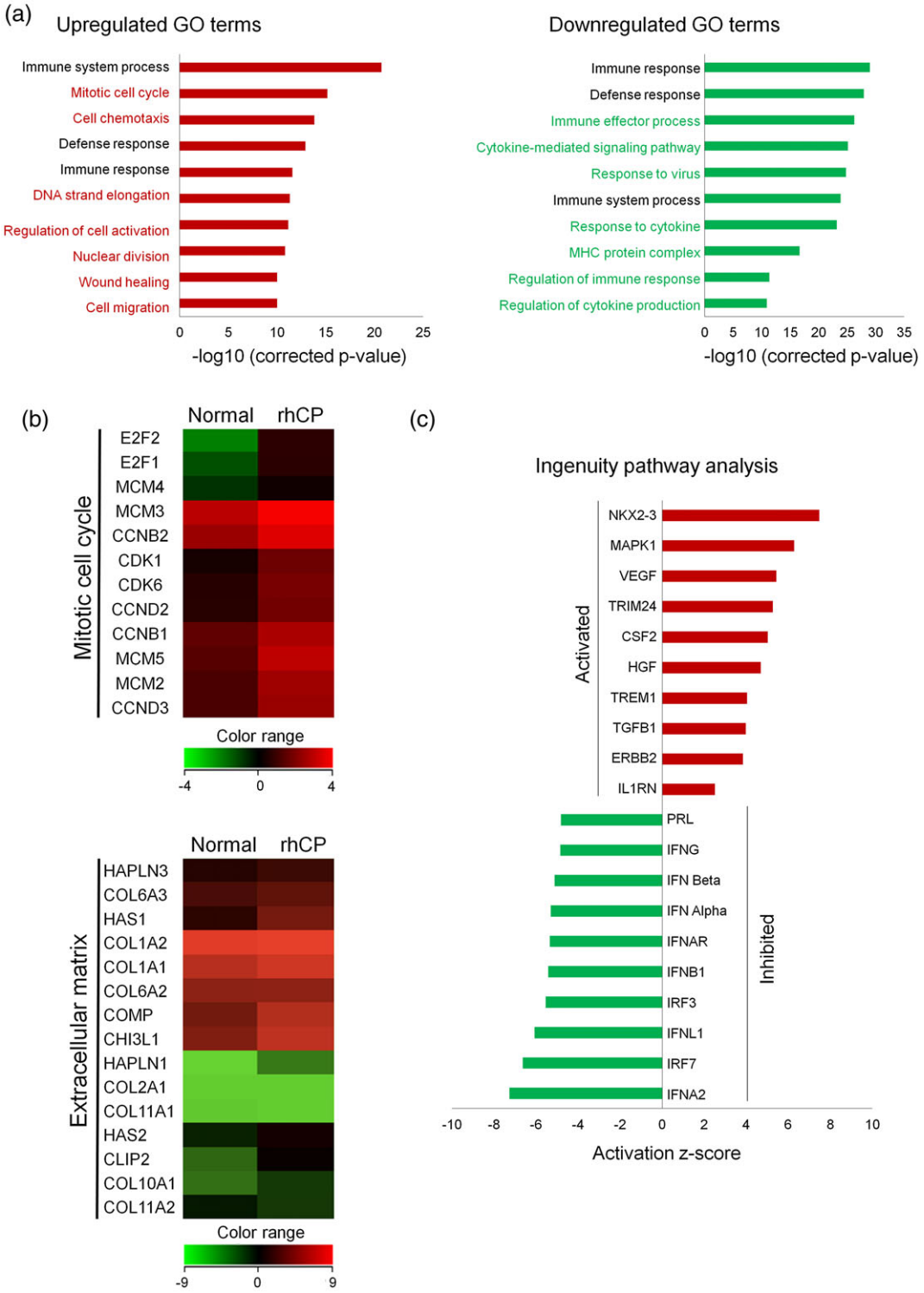

FIGURE 2 Microarray analyses of hASCs cultured on rhCP and on a normal dish (a) summary of the functional categories of genes significantly changed in response to rhCP. Analyses were performed individually on 1195 or 987 doubly upregulated or downregulated genes by rhCP, respectively, using GeneSpring GX. GO terms in black text appeared in both upregulated and downregulated sets. (b) Heat maps indicated that genes related to mitotic cell cycle and extracellular matrix synthesis were upregulated in hASCs cultured on rhCP compared with those cultured on a normal dish. (c) Top 10 pathways activated or inhibited in hASCs cultured on rhCP [Colour figure can be viewed at wileyonlinelibrary.com]

some growth factors/cytokines such as vascular endothelial growth factor (VEGF), hepatocyte growth factor, and transforming growth factor beta were upregulated (Figure 2c), although gene expression of these growth factors was not upregulated (Figure S1). Therefore, it was suggested that rhCP activated the same signaling pathways as those growth factors-dependent receptors. This theory is further supported by the observed upregulation of EC markers such as CD31/PECAM1, CD146/MCAM, VWF, Flk-1/KDR, and Flt-1 (Table 1), which indicates activation of angiogenesis and/or differentiation of hASCs into ECs.

TABLE 1 Gene expression of endothelial cell markers verified by qRT-PCR

Gene	Expression fold-change (log2)		R/N ratio
	Normal	rhCP	
CD31/PECAM1	−0.37	0.5	1.84
CD144/CDH5	−2.72	−2.97	0.84
CD146/MCAM	−2.73	0.25	7.89
VWF	−1.91	0.14	4.13
tie2/TEK	−1.93	−2.4	0.72
Flk-1/KDR	−6.58	−4.73	3.6
Flt-1	−7.15	−5.88	2.4

Note. R; rhCP, N; Normal. rhCP = recombinant human collagen peptide.

All microarray data obtained from our gene expression analyses were deposited with the National Center for Biotechnology Information Gene Expression Omnibus Database (accession no. GSE85129).

3.3 | Wound healing in irradiated tissue models

Four weeks after treatment with 20 Gy, the dorsal skin of the nude mice showed discolouration and loss of elasticity (Figure 3a), and histological analysis by Mallory-Azan staining revealed obliteration of small vessels, subcutaneous fibrosis, and disappearance of cutaneous appendages (Figure 3b). These histologic findings are typical of chronic post-irradiation skin damage.

The therapeutic effects of cells and the rhCP scaffolds were then investigated. Wound healing in the rhCP with hASCs and HUVECs group was best, whereas the rhCP with hASCs and rhCP with PBS groups demonstrated faster and better wound healing, in this order, compared with the controls (Figure 3c,d). The wounds of the rhCP with hASCs and HUVECs group were almost completely healed by Day 14, whereas those of the rhCP with hASCs group were closed on Day 18. In contrast, the wounds of the rhCP with PBS group and the control group had not yet closed by Day 18. Wound sizes on Day 10 in the hASCs and HUVECs with rhCP group, hASCs with rhCP group, rhCP with PBS group, and control group were 7.3%, 19.7%, 36.3%, and 50.3% of original size, respectively. Significant differences were observed between all groups on Day 10 ($p < .05$), suggesting that hASCs, HUVECs, and the rhCP scaffold positively influenced the wound healing process.

4 | DISCUSSION

Finding potent regenerative therapies that can accelerate and support tissue repair in stem cell-depleted challenging conditions is an important task. In this study, we used rhCP sponge, a novel modality to retain and deliver hASCs and/or ECs into damaged tissues. The rhCP, unlike most of naturally based materials of animal origin, is uniquely designed for potential clinical use that can avoid immunological reactions, offers additional integrin-binding motifs, and is expected to enhance therapeutic functions of cells loaded onto the scaffold.

Initial in vitro experiments showed that hASCs can be successfully adhered, survived, and proliferated on the rhCP scaffolds. However, histology and SEM images obtained after 5 days of culture suggested that hASCs could not travel very deep in the rhCP scaffolds within a short period despite the size (100 μm) of micropores. This is suggested to be due to, as the microphotographic images showed, lack of communicating holes between micropores. Accordingly, we applied the rhCP scaffold onto wounds upside down in the in vivo experiments. Structural modifications will be needed to make communicating holes and improve cell distribution in the rhCP scaffold.

Microarray assays indicated upregulated gene expression related to cell cycle and wound healing (especially ECM) in hASCs cultured with rhCP, suggesting that rhCP induces stem cell activation-like responses such as proliferation, migration, and ECM production by hASCs. Pathway analyses then provided further insights into the rhCP scaffold effects on hASCs. These analyses revealed gene expression patterns for inflammatory suppression, cell growth promotion, and, interestingly, increased signals by growth factors/cytokines, including VEGF, hepatocyte growth factor, and transforming growth factor beta, although gene expression of these factors was not actually upregulated.

These findings suggest that the rhCP scaffold induced activation of the signaling pathways downstream of these factors, which play major roles in angiogenesis and tissue regeneration. Integrins were reported to directly activate growth factor receptors by a growth-factor-independent receptor signaling pathways; for example, epidermal growth factor (EGF) receptor phosphorylation can be induced by integrins in the absence of EGF (Moro et al., 1998). In addition, integrin activation can enhance growth factor-dependent receptor signaling, as collaborative activation. Integrins may gather some signaling proteins to create an environment to help some growth factor receptors for their interaction with downstream signaling molecules (Yamada & Even-Ram, 2002), including EGF receptor and VEGF receptor (Ivaska & Heino, 2011). Therefore, it is suggested that the signaling pathways of VEGF receptor were activated by the rhCP containing multiple RGD motifs. Thus, hASCs appeared to be activated to prepare themselves for wound healing and to partly differentiate toward EC as suggested by upregulated gene expression of EC markers.

Based on these findings, rhCP appears to be not only a cell loading material but also a functional cell scaffold that enhances hASC wound healing potential, stimulating the hASCs as if they were already placed on injured tissue during the culture period and priming their responses. This observed biological activity of rhCP is probably due to its integrin binding specificity. The detailed mechanism of rhCP stimulation on cell function, including cell surface receptors and/or signaling pathways activated, was not fully identified in this study, which is a limitation of this study and should be clarified in future studies.

Another limitation of these findings, however, is that the array used only one sample from each group. Using a mice model with impaired wound healing of irradiated tissue, we then confirmed the enhanced therapeutic potential of cells cultured on rhCP for revitalizing the pathological tissue and promoting wound healing. Skin ulcers in irradiated tissue are generally refractory to healing, mainly due to stem cell depletion, ischemia, and fibrosis, and these characteristics make such wounds a reasonable therapeutic target for regenerating/revitalizing cell therapy. Among the four experimental groups, the rhCP with hASCs and HUVECs achieved the fastest and greatest healing quality, even better than the rhCP with hASCs only group. In addition

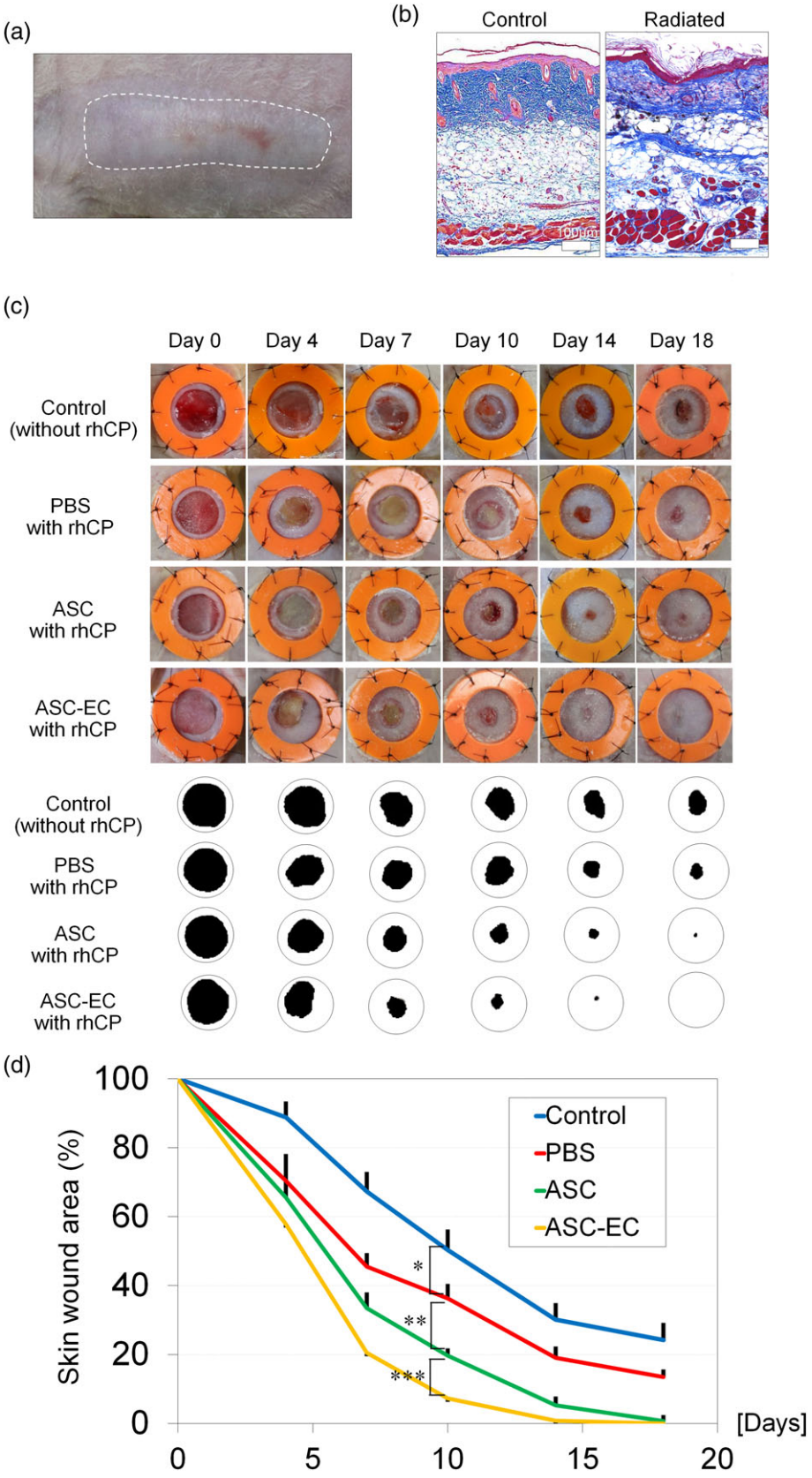

FIGURE 3 Therapeutic mouse model of wound healing in radiated tissue (a) appearance before wound creation. This model underwent 20 Gy of radiation on the back 4 weeks ago and resulted in macroscopic tissue atrophy and fibrosis. (b) Histological analysis revealed fibrosis and disappearance of cutaneous appendages. (c) Representative photos of cutaneous wounds (6 mm) created on the irradiated dorsal areas of mice (n = 3 in each group), with 18-day follow-up. Ulcerated surface areas were determined via digital software. (d) Comparison of wound surface areas (measured digitally). Significantly reduced ulcer sizes were observed between all groups on Day 10. Bonferroni-corrected $^{*}p$ = .018, $^{**}p$ = .007, and $^{***}p$ = .037, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

to previous works that demonstrated function of ECs in both of angiogenesis and inosculation with the capillaries of the host (Auxenfans et al., 2012), emerging evidence supports the use of hASCs and ECs in combination, as interactive signaling between hASCs and ECs that may enhance tissue development processes (Lin et al., 2014). Although this study lacked a group of rhCP with HUVECs alone, a prior study found that dual-cell transplantation of hASCs and ECs formed new vascular networks with much higher density and complexity than transplantation of either hASCs or ECs alone (Traktuev et al., 2009). Human hASCs were highly effective in accelerating repair of irradiated wounds as shown by the difference between the rhCP with hASC group and the rhCP with PBS only group ($p = .007$). This effect on wound healing has been proposed to result from the ability of these cells to enhance the remodeling capacity of irradiated wound bed with their multipotency, secretion of trophic factors, and immune-modulating capacity (Butala et al., 2012; Hong et al., 2010; Kuo et al., 2011; Mashiko & Yoshimura, 2015; Phinney & Prockop, 2007). The rhCP with PBS only group also showed better wound healing than the control group (without rhCP), indicating the therapeutic potential of the rhCP itself: via regulation of biological processes such as cell recruitment, growth, and differentiation in the applied tissues. These findings suggest that rhCP can work not only as a functional cell transplantation substrate but also a topical biomaterial dressing for impaired wound healing.

As described above, the rhCP used in this study is a product designed based on peptides of human collagen type I, has multiple RGD integrin-binding motifs, and was herein shown to be a promising biomaterial which enhances hASC function. Moreover, in contrast to natural collagen, rhCP can be massively manufactured with genetically modified yeast, clinically utilized without any concerns about immunological reaction/rejection, and thus can be considered as a reproducible and consistent approach for wound repair and other applications. There are several previous publications on the same rhCP, in the different forms such as microspheres (Parvizi et al., 2016; Tuin, Kluijtmans, Bouwstra, Harmsen, & Van Luyn, 2010) or gels (Nakamura & Tabata, 2010; Tuin et al., 2012) for injection use, and for the different therapeutic targets such as nephritis (Nakamura & Tabata, 2010) or bone regeneration (Fernandes Patrício, Panseri, Sandri, Tampieri, & Sprio, 2017; Ramírez-Rodríguez et al., 2016). However, this is the first report on the porous sponge of rhCP that offers a simple and practical regenerating approach for wound healing. The rhCP sponge can be combined with ASCs and ECs, both of which can be abundantly obtained from liposuction aspirates with minimal physical morbidity.

5 | CONCLUSION

We successfully cultured hASCs on rhCP scaffolds with good cell adhesion and viability. Gene analysis of these cultured hASCs demonstrated enhanced cell proliferation and wound healing properties. In a mouse impaired wound healing model (irradiated tissue), we found that application of precultured hASCs and HUVECs on rhCP scaffolds accelerated wound healing compared with rhCP scaffold alone, rhCP scaffold with hASCs only, and control wound treatment (no scaffolds nor cells). These results suggest that the rhCP scaffold is a promising therapeutic tool that may provide an optimal cell culture and delivery strategy without immunologic or safety concerns in order to restore healing and regenerating capacity of stem cell-depleted pathological tissues.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

T.M. performed the conception and design, collection, and/or assembly of data; data analysis and interpretation; and manuscript writing. H.T. performed the collection and/or assembly of data, data analysis, and interpretation. S.-H.W. performed the collection and/or assembly of data. K.K. performed the collection and/or assembly of data. J.F. performed the collection and/or assembly of data. K.T. performed the collection and/or assembly of data. M.N. performed the data analysis and interpretation. R.A. performed the collection and/or assembly of data. A.S. performed the collection and/or assembly of data, data analysis, and interpretation. K.H. performed the data analysis and interpretation, administrative support. A.K. performed the collection and/or assembly of data, data analysis and interpretation, manuscript writing. T.T. performed the financial support, administrative support. K.Y. performed the conception and design; data analysis and interpretation; financial support; administrative support; final approval of manuscript; and manuscript writing.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Supplemental Figure S1: Comparative gene expression of hASCs cultured on rhCP or on a normal dish. Cell cycle genes and wound healing genes were upregulated in rhCP group. Some of the immune response genes were upregulated, while others of those were downregulated. Pro- or anti-inflammatory genes and angiogenesis-related genes were not differentially regulated between hASCs on rhCP and those on a normal dish.

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