

Nano-Porous Silica Aerogels as Promising Biomaterials for Oral Drug Delivery of Paclitaxel

Xiaoxiao Wang^{1,2,†}, Jinmei Wang^{2,3,†}, Sishen Feng⁴, Zhian Zhang², Chao Wu², Xuxu Zhang^{2,*}, and Feiyu Kang^{1,5,6,*}

¹Shenzhen Environmental Science and New Energy Technology Engineering Laboratory, Tsinghua-Berkeley Shenzhen Institute (TBSI), Tsinghua University, Shenzhen 518055, People's Republic of China

²Division of Life and Health Sciences, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, People's Republic of China

³School of Mechanical Engineering, Dongguan University of Technology, Dongguan 523808, People's Republic of China

⁴Department of Chemical & Biomolecular Engineering, National University of Singapore, Singapore 117576, Singapore

⁵Division of Energy and Environment, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, People's Republic of China

⁶School of Materials Science and Engineering, Tsinghua University, Beijing 100084, People's Republic of China

Not only dose oral administration have the highest safety, convenience and patient compliance, but also can improve patients' quality of life and reduce health care costs in many cases when compared to the parenteral route. However, many drugs, especially most anticancer drugs such as paclitaxel (PTX), are not orally bioavailable, which is attributed to low aqueous solubility, poor intestinal permeability, and the high level of P-glycoprotein (P-gp) efflux. In this study, we developed a nano-porous silica aerogel delivery system for oral administration of PTX that improved the bioavailability, reduced the side effects of the drug and inhibited tumor growth. The advantages of nano-porous silica aerogel include very high porosities, vast specific surface areas, high drug-loading rate, high biological safety, excellent biocompatibility, and biological inertia, which can also be applied to a variety of drugs. The aerogel delivery system is a universal pharmaceutical system that has huge potential in the field of pharmacy.

KEYWORDS: Amorphous Solid Dispersions, Cancer Chemotherapy, Bioavailability, Oral Delivery, Paclitaxel, Silica Aerogel.

INTRODUCTION

With the introduction of extensive application of combinatorial chemistry and high-throughput screening technology of novel drugs in research and development, the generation of new compound entities is growing rapidly. However, in many new compounds with physiological activity, hydrophobic compounds are increasing and the proportion may be as high as 40%.¹ Moreover, the proportion of chemically synthesized candidate compounds is as high as 60%, and an estimated 90% of drugs that are currently in development can be classified as poorly soluble.^{2,3} In the development stage, up to 40%–70% of the compounds cannot produce a sufficient curative effect due to insufficient solubility. Therefore, the research and development

of novel promising drugs takes time, requires a high input, and the risk is high.⁴ Many candidate compounds cannot be orally absorbed and are less suitable to being directly prepared for injections due to the problem of solubility, therefore termination of the development is high, leading to economic loss and a waste of resources.⁵

The pharmaceutical industry has developed multiple methods for increasing the solubility of poorly soluble drugs.^{6–9} Traditionally, salt formation was preferred by medicinal and synthetic chemists.¹⁰ Unfortunately, only 20–30% of novel molecules can easily form salts, therefore for 70–80% of those entities another route must be identified to improve solubility.² The introduction of nano-technologies provides broad prospects for the development of pharmaceutical compounds. In recent decades, various nano-drug delivery systems, such as emulsions,^{11–17} solid lipid nanoparticles,^{18–21} micelles,^{22–28} liposomes,^{29–31} nanoparticles of functional polymers,^{32–41} nanocrystals,^{42–45} and nano-drug-hydrogel composites⁹

* Authors to whom correspondence should be addressed.

Emails: zhangxx@sz.tsinghua.edu.cn, fykang@sz.tsinghua.edu.cn

[†]These two authors contributed equally to this work.

Received: 19 July 2018

Accepted: 30 September 2018

SCIENTIFIC REPORTS

OPEN

TSA restores hair follicle-inductive capacity of skin-derived precursors

Ling Guo^{1,3}, Xiaoxiao Wang^{1,2,3}, Jifan Yuan³, Meishu Zhu⁴, Xiaobing Fu^{5,6}, Ren-He Xu⁷, Chuanyue Wu⁸ & Yaojiong Wu^{1,2}

Received: 11 July 2018

Accepted: 21 January 2019

Published online: 27 February 2019

The genesis of the hair follicle relies on signals derived from mesenchymal cells in the dermis during skin morphogenesis and regeneration. Multipotent skin-derived precursors (SKPs), which exhibit long term proliferation potential when being cultured in spheroids, have been shown to induce hair genesis and hair follicle regeneration in mice, implying a therapeutic potential of SKPs in hair follicle regeneration and bioengineering. However, the hair-inductive property of SKPs declines progressively upon *ex vivo* culture expansion, suggesting that the expressions of the genes responsible for hair induction are epigenetically unstable. In this study, we found that TSA markedly alleviated culture expansion induced SKP senescence, increased the expression and activity of alkaline phosphatase (AP) in the cells and importantly restored the hair inductive capacity of SKPs. TSA increased the acetylation level of histone H3, including the K19/14 sites in the promoter regions of bone morphogenetic proteins (BMPs) genes, which were associated with elevated gene expression and BMP signaling activity, suggesting a potential attribution of BMP pathway in TSA induced recovery of the hair inductive capacity of SKPs.

The genesis of the hair follicle relies on signals derived from mesenchymal cells in the dermis during skin morphogenesis and regeneration^{1–3}. Previous studies indicate that dermal papilla (DP) cells derived from the hair follicle are able to induce hair follicle formation^{4–6}. However, the application of DP cells in tissue engineering has been limited by their availability. The cells can only be isolated manually from large hair follicles in the scalp and their hair-inductive property diminishes markedly upon culture expansion^{7,8}. Intriguingly, multipotent skin-derived precursors (SKPs) have recently been shown to induce hair follicle formation. SKPs express Sox2 and nestin, and exhibit long term proliferation potential when being cultured in spheroids^{3,9,10}. When subcutaneously injected in mice the cells were found to incorporate into the DP and induce hair genesis¹⁰, and when transplanted in combination with epidermal stem cells into excisional wounds in mice, SKPs induced *de novo* hair genesis¹¹. These results imply a potential application of SKPs in hair follicle regeneration and bioengineering. However, the hair-inductive property of SKPs declines progressively upon *ex vivo* culture expansion^{11,12}, suggesting that the expression of the genes responsible for hair induction are epigenetically unstable.

Trichostatin A (TSA) is a potent and specific inhibitor of a histone deacetylase (HDAC) activity^{13,14}. It selectively inhibits the class I and II, but not class III, mammalian HDAC families of enzymes¹⁵. Acetylation of K9 and K14 in histone H3 is required for the recruitment of TFIID¹⁶, and TFIID binding to the promoter causes DNA bending and downstream translocation of the SWI/SNF-modified nucleosome, which allows the initiation of transcription¹⁷. In our previous study, we have proved that the altered expression of these genes was closely associated with epigenetic dysregulation of histone H3 acetylation in K9 and K14¹⁸. It has been shown previously that

¹State Key Laboratory of Chemical Oncogenomics, and the the Shenzhen Key Laboratory of Health Sciences and Technology, Graduate School at Shenzhen, Tsinghua University, Shenzhen, China. ²Tsinghua-Berkeley Shenzhen Institute (TBSI), Tsinghua University, Shenzhen, China. ³Guangdong Provincial Key Laboratory of Cell Microenvironment and Disease Research, Shenzhen Key Laboratory of Cell Microenvironment, and Department of Biology, Academy for Advanced Interdisciplinary Studies, Southern University of Science and Technology, Shenzhen, China. ⁴Shenzhen Second People's Hospital (The First Hospital Affiliated to Shenzhen University), Shenzhen, China. ⁵Wound Healing and Cell Biology Laboratory, Institute of Basic Medical Science, Chinese PLA General Hospital, Beijing, China. ⁶Stem Cell and Tissue Regeneration Laboratory, The First Affiliated Hospital, General Hospital of PLA, Beijing, China. ⁷University of Macau, Institute of Translational Medicine, and Centre of Reproduction, Development and Aging, Faculty of Health Sciences, Taipa, Macau, China. ⁸Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15261, USA. Ling Guo, Xiaoxiao Wang and Jifan Yuan contributed equally. Correspondence and requests for materials should be addressed to C.W. (email: carywu@pitt.edu) or Y.W. (email: wu.yaojiong@sz.tsinghua.edu.cn)



Isolation and Cultivation of Skin-Derived Precursors

Xiaoxiao Wang, Shiyang Dong, and Yaojiong Wu

Abstract

Skin-derived precursors (SKPs) have been shown recently to be capable of inducing hair genesis and hair follicle regeneration. Here, we describe a protocol for SKP isolation and culture based on our experience and previous publications.

Keywords Cultivation, Isolation, Skin-derived precursor, SKPs

1 Introduction

The formation of hair follicles relies on signals derived from the dermis during skin morphogenesis and regeneration number [1, 2]. Previous studies indicate that dermal papilla (DP) cells in the hair follicle are able to induce hair genesis [3]. However, the application of DP cells is limited by their availability. Recently, multipotent skin-derived precursors (SKPs) have been isolated from the dermis of adult skin of different anatomic sites and shown to be capable of inducing hair genesis and hair follicle regeneration [4–7], implying a potential application as induction cells in hair genesis and skin bioengineering.

2 Materials

2.1 Reagent Preparation

1. Phosphate buffer saline (PBS).
2. DPBS (Corning).
3. Saline.
4. 70% ethanol.
5. 2% (W/V) iodine solution.
6. 100× Penicillin/streptomycin solution (Gibco).
7. Gentamycin.
8. SKP growth medium: DMEM: F12, 3:1 mixture (Corning), supplemented with 20 µl/ml B27, 40 ng/ml basic fibroblast



ELSEVIER



BASIC SCIENCE

Nanomedicine: Nanotechnology, Biology, and Medicine
12 (2016) 2115–2125



nanomedjournal.com

Original Article

Self-assembling peptide hydrogel scaffolds support stem cell-based hair follicle regeneration

Xiaoxiao Wang, PhD^{a,b,1}, Jinmei Wang, PhD^{b,1}, Ling Guo, MD^b, Xusheng Wang, PhD^{a,b}, Haiyan Chen, MS^{a,b,c}, Xiumei Wang, PhD^d, Jianjun Liu, PhD^e, Edward E. Tredget, MD^f, Yaojiong Wu, MD, PhD^{b,c,*}

^aSchool of Life Sciences, Tsinghua University, China

^bThe Shenzhen Key Laboratory of Health Sciences and Technology, Graduate School at Shenzhen, Tsinghua University, China

^cTsinghua-Berkeley Shenzhen Institute (TBSI), Tsinghua University, China

^dSchool of Materials Science and Engineering, Tsinghua University, China

^eMedical Key Laboratory of Health Toxicology of Shenzhen, Shenzhen Center for Disease Control and Prevention, Shenzhen, China

^fWound Healing Research Group, Department of Surgery, University of Alberta, Edmonton, AB, Canada

Received 2 March 2016; accepted 24 May 2016

Abstract

Recent studies show that designer peptide nanofibers can mimic properties of extracellular matrix molecules, promising great potential as scaffold materials for tissue engineering. However, their ability in supporting organogenesis has not been studied. Here we examined the effect of self-assembling peptide hydrogels in supporting skin derived precursors (SKPs) in hair follicle neogenesis. We found that hydrogels formed by RADA16, PRG which contains RGD, and particularly the combination of RADA16 and PRG (RADA-PRG) enhanced SKP proliferation. Notably, the RADA-PRG hydrogel, which exhibited advantages of RADA16 in adequate nanofiber formation and PRG in providing the integrin binding sequence, exhibited superior effects in enhancing SKP survival, expression of hair induction signature genes such as *Akp2* and *Bmp6*, and more importantly *de novo* hair genesis in mice. Thus our results suggest that RADA-PRG may serve as a novel scaffold material for stem cell transplantation and tissue engineering.

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Key words: Peptide hydrogel; RADA16; PRG; Hair follicle neogenesis; Skin derived precursors

Skin is the largest organ in the body, which provides a barrier against infections and fluid loss and has several homeostatic/sensory functions vital to health. Restoration of

skin is of great importance in the treatment of severe skin defect. The limited availability of donor skin has led to the development of tissue engineered skin equivalents as alternatives in burn wound treatment. Engineered skin substitutes consisting of an epidermal substitute of autologous keratinocytes and a dermal analog of bovine collagen–glycosaminoglycan sponge populated with autologous fibroblasts are able to form a functional epidermal barrier, but not the epidermal appendage including the hair follicle, sebaceous gland and sweat gland.^{1,2}

Skin appendages, which extend deeply into the dermis, are derived from a single layer of multipotent progenitors during skin morphogenesis.³ Recent studies indicate that stem cells capable of forming *de novo* hair follicles reside in the skin; however, this process relies on appropriate signals derived from dermal cells.^{4–6} Previous studies indicate that dermal papilla (DP) cells derived from the hair follicle are able to induce hair follicle genesis,^{7–9} but the application of DP cells in the

Author contributions: X.W., J.W.: designed and performed experiments, and performed data analysis; L.G., X.W., H.C.: performed experiments; X.W., J.L.: provided materials and designed experiments; E.T.: designed experiments; Y.W.: designed experiments and wrote the manuscript.

This work was supported by grants from Natural Science Foundation of China (31371404, 31571429), Natural Science Foundation of Guangdong (2015A030311041), and Shenzhen Science and Technology Innovation Committee (JCYJ20140417115840279, GJHZ20150316160614842 and JCYJ20160301150838144).

Conflict of Interest and Disclosure: N/A.

*Corresponding author at: The Shenzhen Key Laboratory of Health Sciences and Technology, Graduate School at Shenzhen, Tsinghua University, Shenzhen, China.

E-mail address: wu.yaojiong@sz.tsinghua.edu.cn (Y. Wu).

¹ These authors contributed equally to this work.

<http://dx.doi.org/10.1016/j.nano.2016.05.021>

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Hair Follicle and Sebaceous Gland De Novo Regeneration With Cultured Epidermal Stem Cells and Skin-Derived Precursors

XIAOXIAO WANG,^{a,b,*} XUSHENG WANG,^{a,b,*} JIANJUN LIU,^c TING CAI,^{a,b} LING GUO,^b SHUJUAN WANG,^d JINMEI WANG,^b YANPEI CAO,^e JIANFENG GE,^{a,b} YUYANG JIANG,^d EDWARD E. TREDGET,^f MENGJUN CAO,^g YAOJIONG WU^{b,h}

Key Words. Epidermal stem cells • Skin-derived precursors • Hair follicle regeneration • BMP4 • Sebaceous glands

ABSTRACT

Stem cell-based organ regeneration is purported to enable the replacement of impaired organs in the foreseeable future. Here, we demonstrated that a combination of cultured epidermal stem cells (Epi-SCs) derived from the epidermis and skin-derived precursors (SKPs) was capable of reconstituting functional hair follicles and sebaceous glands (SG). When Epi-SCs and SKPs were mixed in a hydrogel and implanted into an excisional wound in nude mice, the Epi-SCs formed de novo epidermis along with hair follicles, and SKPs contributed to dermal papilla in the neogenic hair follicles. Notably, a combination of culture-expanded Epi-SCs and SKPs derived from the adult human scalp were sufficient to generate hair follicles and hair. Bone morphogenetic protein 4, but not Wnts, sustained the expression of alkaline phosphatase in SKPs in vitro and the hair follicle-inductive property in vivo when SKPs were engrafted with neonatal epidermal cells into excisional wounds. In addition, Epi-SCs were capable of differentiating into sebocytes and formed de novo SGs, which excreted lipids as do normal SGs. Thus our results indicate that cultured Epi-SCs and SKPs are sufficient to generate de novo hair follicles and SGs, implying great potential to develop novel bioengineered skin substitutes with appendage genesis capacity. *STEM CELLS TRANSLATIONAL MEDICINE* 2016;5:1695–1706

SIGNIFICANCE

In postpartum humans, skin appendages lost in injury are not regenerated, despite the considerable achievement made in skin bioengineering. In this study, transplantation of a combination of culture-expanded epidermal stem cells and skin-derived progenitors from mice and adult humans led to de novo regeneration of functional hair follicles and sebaceous glands. The data provide transferable knowledge for the development of novel bioengineered skin substitutes with epidermal appendage regeneration capacity.

INTRODUCTION

Tissue engineering is emerging as a significant potential solution for tissue and organ failure, whereby tissue and organ mimics that are fully functional are implanted to replace the failed tissue and organ [1]. Adult skin consists of a keratinized stratified epidermis and an underlying layer of dermis. Hair follicles, sebaceous glands (SGs), and sweat glands, the appendages of the skin, are derived from a single layer of multipotent progenitors during skin morphogenesis [2]. In postpartum humans, however, deep injuries to the skin heal by scar formation but not by regeneration; epidermal appendages lost at the injury site do not regenerate [3].

It has been a challenge to regenerate skin appendages with defined cells. More than 10 years

have passed since the development of cultured skin substitute (CSS), which consists of cultured autologous epidermis and dermal fibroblasts. The CSS is capable of forming an epidermal layer (barrier function) but does not regenerate the appendages [4]; thereby the structure and function of the skin are not fully restored. A bioengineered skin substitute capable of regenerating the epidermis and the epidermal appendages has been an objective to achieve.

Several populations of stem cells have been identified in the skin. Epidermal stem cells (Epi-SCs) in the basal layer epidermis—which express high levels of cytokeratin (CK)5, CK14, CD29 (integrin β 1), and CD49f (integrin α 6) [5]—constantly provide new keratinocytes to the epidermis, implying great proliferation potential. Neonatal mouse

^aSchool of Life Sciences,

^bShenzhen Key Laboratory of Health Sciences and Technology, Graduate School at Shenzhen, ^dState Key Laboratory Breeding Base—Shenzhen Key Laboratory of Chemical Biology, Graduate School at Shenzhen, and ^hTsinghua-Berkeley Shenzhen Institute, Tsinghua University, Beijing, People's Republic of China; ^cMedical Key Laboratory of Health Toxicology of Shenzhen, Shenzhen Center for Disease Control and Prevention, Shenzhen, People's Republic of China; ^eClinical Research Center, University College Dublin, Belfield, Dublin, Ireland; ^fWound Healing Research Group, Department of Surgery, University of Alberta, Edmonton, Alberta, Canada; ^gShenzhen Fuhua Aesthetic Hospital, Shenzhen, People's Republic of China

*Contributed equally.

Correspondence: Yaojiong Wu, M.D., Ph.D., L406A, Tsinghua Campus, University Town, Shenzhen, People's Republic of China. Telephone: 755-2603-6348; E-Mail: wu.yaojiong@sz.tsinghua.edu.cn

Received December 14, 2015; accepted for publication May 12, 2016; published Online First on July 25, 2016.

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1066-5099/2016/\$20.00/0

<http://dx.doi.org/10.5966/sctm.2015-0397>