UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF CONDITIONED MEDIUM DERIVED FROM RAT AMNIOTIC FLUID STEM CELLS SUITABLE FOR RESTORATION OF DIABETIC WOUND

HASFAR AMYNURLIYANA BINTI ABDUL GHOFAR

IB 2018 13

DEVELOPMENT OF CONDITIONED MEDIUM DERIVED FROM RAT AMNIOTIC FLUID STEM CELLS SUITABLE FOR RESTORATION OF DIABETIC WOUND

By

HASFAR AMYNURLIYANA BINTI ABDUL GHOFAR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

October 2017

DEDICATION

This thesis is dedicated to my beloved mother Mrs. Hasemah Omar, my father Mr. Abdul Ghofar Abdul Mubin, my little sisters (Miss Hasfar Syazwani and Miss Hasfar Syafiqah), my siblings, my relatives and friends more like family.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

DEVELOPMENT OF CONDITIONED MEDIUM DERIVED FROM RAT AMNIOTIC FLUID STEM CELLS SUITABLE FOR RESTORATION OF DIABETIC WOUND

By

HASFAR AMYNURLIYANA BINTI ABDUL GHOFAR

October 2017

Chair: Sharida Fakurazi, PhD
Institute: Bioscience

Wound in diabetics is a slow healing and chronic process. Stem cells have been reported to mobilize the wound site, by secreting cellular growth factors and cytokines during their cultivation by promoting angiogenesis and remodeling of extracellular matrix (ECM), creating a favorable local environment for wound healing. The conditioned medium (CM) is a medium containing growth factors and cytokines cultivated in viable optimum environment for a certain period of time. These factors may promote the growth of new cells especially in wound microenvironment. Therefore, this work is conducted to establish and characterize rat amniotic fluid stem cells conditioned medium (rAFSC-CM) that is suitable for *in vitro* diabetic wound healing model using diabetic human dermal fibroblasts (HDF-D). To achieve this aim, a suitable number of rAFSCs density, which has been isolated, identified, and confirmed were used in the preparation of CM. The cells density at 0.25×10^6 cells/mL was used to prepare the CM with the presence of leukemia inhibitory factor (LIF+) and absence of LIF (LIF-). Cell proliferation assays and scratch test assays were conducted to substantiate the ability of rAFSCs-CM in enhancing the Diabetic Human Dermal Fibroblast (HDF-D) cell proliferation and migrations. The expression of molecular markers such as TGF-β1, VEGF, IL-6, IL-1β, and TNF-α in CM were identified using Enzyme Linked Immunosorbent Assay (ELISA) and Western's blotting technique. The results revealed rAFSC-CM has significantly improved the migration, viability, and proliferation of HDF-D cells. ELISA and Western Blot indicated that the rAFSCs-CM contains various growth factors that are known to be important in wound healing including TGF-β1, VEGF, IL-6, IL-1β, and TNF-α. From the data observed, it was suggested that low concentration (25%) of rAFSC-CM with the absence of LIF (LIF-) showed the most potential CM with a good potential of proliferation and migration activity. As a conclusion, this study showed that rAFSCs secreted high levels of cytokines and growth factors that enhance the wound healing, and further studies using specific mechanism experiments *in vivo* are needed to uncover and improve its effectiveness in cell-free therapies to encourage the healing of diabetic skin wound.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PEMBANGUNAN MEDIUM TERLAZIM DARIPADA SEL TUNJANG BENDALIR AMNION TIKUS SESUAI UNTUK PEMULIHAN LUKA KULIT PESAKIT KENCING MANIS

Oleh

HASFAR AMYNURLIYANA BINTI ABDUL GHOFAR

Oktober 2017

Pengerusi: Sharida Fakurazi, PhD
Institut: Biosains

Luka diabetes merupakan proses penyembuhan yang perlahan dan kronik. Sel tunjang telah dilaporkan menggerakkan tapak luka dengan merembeskan faktor pertumbuhan sel dan sitokin semasa kultivasi dengan menggalakkan angiogenesis dan pembentukan semula matriks luar sel (ECM), Mewujudkan persekitaran setempat yang menggalakkan bagi penyembuhan luka. Medium terlazim (CM) ialah satu medium yang mengandungi faktor pertumbuhan dan sitokin yang dibangunkan dalam persekitaran optimum yang berupaya hidup bagi jangka masa tertentu. Faktor-faktor ini boleh menggalakkan pertumbuhan sel-sel baru terutamanya dalam mikropersekitaran luka. Oleh itu, kajian ini dijalankan untuk membentuk dan mencirikan medium terlazim Sel Tunjang Bendalir Amnion Tikus (rAFSC-CM) yang sesuai untuk model *in vitro* penyembuhan luka diabetes menggunakan fibroblas diabetes derma manusia (HDF-D). Untuk mencapai matlamat ini, bilangan ketumpatan rAFSCs bersesuaian, yang telah diasingkan, dikenal pasti, dan disahkan telah digunakan dalam persediaan CM. Ketumpatan sel-sel pada 0.25×10^6 sel/mL digunakan untuk menyediakan CM dengan penambahan faktor rencatan leukemia (LIF+) dan tanpa LIF (LIF-). Assai proliferasi sel dan assai ujian calar migrasi telah dijalankan untuk membuktikan keupayaan rAFSCs-CM dalam meningkatkan proliferasi dan migrasi sel Diabetes Fibroblas Derma Manusia (HDF-D). Ekspresi penanda molekular seperti TGF-β1, VEGF, IL-6, IL-1β, dan TNF-α dalam CM telah dikenalpasti menggunakan Asai Imunoserapan Terangkai Enzim (ELISA) dan teknik pemendapan Western. Hasil kajian mendedahkan rAFSC-CM telah dengan ketara meningkatkan penghijrahan, kebolehhidupan, dan proliferasi sel HDF-D. ELISA dan pemendapan Western menunjukkan bahawa rAFSCs-CM mengandungi pelbagai faktor-faktor pertumbuhan yang diketahui penting dalam penyembuhan luka termasuk TGF-β1, VEGF, IL-6, IL-1β, dan TNF-α. Daripada data yang diperhatikan, adalah disarankan bahawa rAFSC-CM tanpa LIF (LIF-) berkepekatan rendah (25%) mempamerkan CM yang paling berpotensi dengan potensi proliferasi dan aktiviti migrasi. Kesimpulannya, kajian ini menunjukkan rAFSCs merembeskan tahap sitokin dan faktor pertumbuhan yang tinggi yang boleh meningkatkan penyembuhan

luka, dan kajian lanjut menggunakan mekanisme eksperimen khusus *in vivo* diperlukan untuk mendedahkan dan meningkatkan keberkesanannya dalam terapi-terapi tanpa sel dalam menggalakkan penyembuhan luka kulit pesakit kencing manis.

ACKNOWLEDGEMENTS

First and foremost, I praise Allah, the almighty for providing me this opportunity and granting me the capability to proceed successfully in my academic pursuit. I also appreciate and thank everyone for their support and blessings throughout this journey.

I would like to thank,

- My parents, for their trust in me and permitting me to en route towards my striving goals. My father, Mr. Abdul Ghofar Abdul Mubin and my mother Mrs. Hasemah Omar who raised me with love, support, caring, affection and always there for me in every hard and easy time. I would also like to extend my thanks to my sisters Miss Hasfar Syazwani and Miss Hasfar Syafiqah and all my family members for their understandings and being supportive throughout my study period. You guys are the most caring and affectionate people I know and the blessed life I have would not have been possible without your love.

- And sincerely thank my supervisor, Associate Prof. Datin Dr. Hajjah Sharida Fakurazi, for allowing me to conduct this research under her supervision. I am really grateful for her confidence and the freedom provided by her during this work. Her eternal energy and eagerness in research extremely motivated me in my studies and helped me to be more productive than I could have imagined. Her guidance, inspiration and willingness to treat her research team as a part of her family members, allowed me to move at ease with her. Her art of understanding the pulse of every student under her supervision still amazes me, and she provided appropriate breaks when we desperately required. Her bright ideas and input have helped me in planning the experiments, in analyzing data and in accelerating this thesis preparation. Without articulate and illuminating instructions from her, this thesis would not have reached its present form.

- Also, I extend my deepest gratitude to my co-supervisor, Dr. Shariza Nordin for her eminent support, constructive criticism and guidance by sharing her expertise knowledge in the field of stem cells, cell culture and biochemical techniques pave way to accomplish my work successfully. Her scientific excitement inspired me in the most important moments of making right decisions and had significantly contributed to this thesis.

- My fellow lab-mates at laboratory of Vaccines and Immunotherapeutic, Institute of Bioscience and Stem Cells Laboratory, Faculty of Medicine and Health Sciences for their stimulating discussions, for the sleepless nights we worked together before deadlines, and for all the fun we have had in the last four years journey. I would like thank mainly my close buddies, Shafinaz and Shila, for their philosophies and

guidance in my life. Also I would extend my thanks to Karthi, Dr.Aminu, Aimi, Sarah for their bounty-less helps and motivations.

- I must also acknowledge the financial support for this project from University Research Grant Scheme (RUGS), Universiti Putra Malaysia, Grant No. 9366700.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Sharida Binti Fakurazi, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Norshariza Binti Nordin, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: _____

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AD-MSCs	Adipose-derived mesenchymal stem cells
AD-MSC-CM	AD-derived-mesenchymal stem cells-conditioned medium
AD-SC	Adipose-derived stem cells
AF	Amniotic fluid
AFSCs	Amniotic fluid stem cells
AFSC-SCs	Amniotic fluid stem-derived stem cells
AF-MSC	Amniotic fluid-derived-derived mesenchymal stem cells
AFSC-CM	Amniotic fluid stem cells-conditioned media
ASCs	Adult stem cells
BMI	Body mass index
BM-MSC	Bone marrow-derived mesenchymal stem cells
BMP/SMAD	Bone morphogenetic proteins/SMAD
BM-SCs	Bone marrow-derived stem cells
BSA	Bovine Serum Albumin
CD29	Cluster of differentiation 29
CD31	Cluster of differentiation 31
CD35	Cluster of differentiation 35
CD44	Cluster of differentiation 44
CD45	Cluster of differentiation 45
CD73	Cluster of differentiation 73
CD90	Cluster of differentiation 90
CD105	Cluster of differentiation 105
CD117	Cluster of differentiation 117
CD133	Cluster of differentiation 133

CM	Conditioned medium
CO_2	Carbon dioxide
DF-1	Dermal fibroblast growth
DMEM	Dulbecco's Modified Eagle's Medium
DMEM/F-12	Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12
EC	Embryo carcinoma cells
ECM	Extracellular matrix
EG	Embryonic germ cells
EGF	Epidermal growth factor
ELISA	Enzyme-Linked Immunosorbent Assay
ESCs	Embryonic stem cells
FACS	Fluorescence Activated Cell Sorting
FBS	Fetal Bovine Serum
FGF-5	Fibroblast growth factor 5
FGF-a, FGF-b	Fibroblast growth factors (acidic and basic)
GMEM	Glasgow Minimum Essential Medium
hAF	Human amniotic fluid
hAFSCs	Human amniotic fluid stem cells
HDF-D	Diabetic human dermal fibroblast
hESCs	Human embryonic stem cells
hSCs	Human stem cells
ICC	Immunocytochemistry
IL-2	Interleukin 2
IL-4	Interleukin 4
IL-6	Interleukin 6

IL-8	Interleukin 8
IL-1β	Interleukin 1 beta
iPSCs	Induced pluripotent stem cells
KGF	Keratinocytes growth factor
LIF	Leukemia Inhibitory Factor
LIF/STAT	Leukemia inhibitory factor/signal transducers and activators of transcription
LIF/STAT 3	Leukemia inhibitory factor/signal transducers and activators of transcription 3
MEM NEAA	Minimum Essential Medium Non-Essential Amino Acids
mESCs	Murine embryonic stem cells
MHCI	Histocompatibility complex I
MHC-II	Histocompatibility complex II
MMPs	Matrix metalloproteinase
MSCs	Mesenchymal stem cells
NANOG	Nanog homeobox
OCT4	Octamer-binding transcription factor 4
PBS	Phosphate Buffered Saline
PBST	Phosphate buffered saline with Tween 20
PDT	Population of doubling time
PDGF	Platelet-derived growth factor
POU	Pit-Oct-Unc
rAFSCs	Rat amniotic fluid stem cells
rAFSCs-CM	Rat amniotic fluid stem cells-conditioned medium
RIPA	Radioimmunoprecipitation Assay
SCs	Stem cells

SC-CM	Stem cells-derived-conditioned medium
SOX2	SRY (sex determining region Y)-box 2
SSEA1	Stage-specific embryonic antigen-1
STAT3	Signal transducer and activator of transcription 3
TERT	Telomerase reverse transcriptase
TGF-β	Transforming growth factor beta
TGF- β1	Transforming growth factor beta 1
TIMP	Tissue inhibitor of metalloproteinase
TNF-α	Tumor necrosis factor alpha
TNFRI	Tumor necrosis factor receptor 1
VEGF	Vascular endothelial growth factor
VEGF-C	Vascular endothelial growth factor C

CHAPTER 1

INTRODUCTION

1.1 Background of study

1.1.1 Wound in diabetes mellitus

Diabetes mellitus (DM) is a chronic disease and the incidence has risen globally to 1.5 million mortality in 2012 (WHO, 2016). The report has indicated that there are 108 million patients with diabetes in 1980 and the number has increased to about 422 million in 2014 where the number has doubled from 4.7% to 8.5% of adult population with diabetes. This disease is a common and affecting both advance and developing countries. Diabetes mellitus can lead to other serious and chronic complications such as infections, neuropathy, cardiovascular disease, and impaired wound healing (Shin & Peterson, 2012). During the lifetime of diabetic patients, 15% of them develop foot ulcers and 85% develop non-healing ulcers that are responsible for the non-traumatic lower extremity amputation (Gulcan et al., 2012). Impaired or delayed wound healing is one of the main reasons which leads to ulcers of lower extremities and has become one of the most challenging complications of DM (Esmaeelinejad et al., 2014; Greenhalgh, 2003). Delayed or improper treatment unfavourably have an effect on the time for wound healing, affect the quality of life, and increasing the burden on patients, their families and carers, society and the health economy (Vowden et al., 2016).

Wound healing is a complex process of cellular and biochemical interactions that involve a variety of cells such as keratinocytes, fibroblasts and endothelial cells. The wound healing process can be categorized into four overlapping phases: hemostasis, inflammation, proliferation, and remodeling (Blumberg et al., 2012; Enoch & Leaper, 2007; Falanga, 2005; Tsourdi et al., 2013). Haemostasis is a process which causes the bleeding to stop that occurs within an hour after injury and is characterised by vasoconstriction and clotting (Tsourdi et al., 2013). Inflammatory phase is characterised by leukocyte migration into the wound (Blumberg et al., 2012). Proliferation phase is characterised by re-epithelialization, neovascularization and the formation of extracellular matrix (ECM) (Blumberg et al., 2012). Proliferation phase overlaps with the inflammatory phase and the most important cells are fibroblasts, which is responsible for initiating angiogenesis, epithelialisation and the formation of collagen. Fibroblasts are essential to wound healing process by secreting, contracts, and remodeling of the extracellular matrix (ECM). Remodeling phase is characterised by further deposition of collagen and a crosslinking in the ECM, in which scar tissue tensile strength (Blumberg et al., 2012). Therefore, any obstacles to fibroblast function are restriction to the normal wound healing and can lead to chronic, difficult to heal wounds. The diabetic wound is characterised by impaired wound healing phases, particularly inflammatory and proliferative phase (Breitbart et al., 2003). Impaired wound healing may be accredited with a defect in normal tissue

response to injury and poor treatment of wounds. Chronic wounds are defined as those which do not appear to follow the normal healing process in less than 4 weeks.

1.1.2 Amniotic fluid stem cells conditioned medium

The amniotic fluid (AF) has protective functions and surrounds the developing fetus in humans and mice, have been shown to contain stem cells (SCs). It can be extracted through the mother's abdomen by insertion of a long, thin needle in a process called amniocentesis which sometimes used to test for genetic diseases including Down Syndrome and generally, it is considered safe for both mother and embryo (Loukogeorgakis et al., 2016).

The amniotic fluid stem cells (AFSCs) are pluripotent and multipotent SCs that are able to differentiate into multiple tissue types that share the same characteristics of both adult and embryonic stem cells (ESCs). The important advantages of AFSCs over most of the known adults SCs sources are their high proliferation rate and differentiation potential into cells of all three embryonic germ layers (Siegel et al., 2007). The cells are reported to be useful in cell therapy and clinical applications (Delo et al., 2006; Rota et al., 2012 and Yang et al., 2013). The advantage of using AFSCs over the ones acquired from embryos is that they evade ethical issues among pro-life activists by obtaining the undifferentiated cells pluripotent lines without harming the foetus or damaging the embryos (Yang et al., 2013). It is proven to be reliable, safe and simple screening tool for a wide variety of developmental and genetic diseases. AFSCs are pluripotent stem cells based on their ability to differentiate into derivatives that represent the three primary germ layers and expression of octamer-binding transcription factor 4 (OCT4), which is a marker for undifferentiated SCs (Kim et al., 2007a; Prusa et al., 2003). Another defining characteristic of pluripotent SCs is the expression of TERT gene, which codes for telomerase reverse transcriptase (Mun-Fun et al., 2015). A study has shown that the human AFSCs (hAFSCs) has a great potential in cosmetic dermatology, especially in the treatment or regeneration of skin injury in considering the clinical efficacy and safety as well as their secretion factors (Yoon et al., 2010).

Conditioned medium (CM) contains secreted factors from SCs derived that referred to as secretome, growth factors and cytokines (Pawitan, 2014). The secreted factors itself may be able to repair tissue in various conditions that involved tissues or organ damage and has a promising prospect to be developed in regenerative medicine (Pawitan, 2014). Moreover the CM have several advantages compared to the SCs itself as the CM can be manufactured, freeze dried, package and transported more easily. There are also no need to match the donor and the recipient to avoid the rejection as it is devoid of cells (Pawitan, 2014).

Human stem cells (hSCs) have raised ethical and political controversies. Adult stem cells (ASCs) and cord blood SCs are multipotent SCs that are widely used in research and clinical care, where less issues are being raised with the use of those

two (Lo & Parham, 2009). However, human embryonic stem cells (hESCs) research have raised ethical and political controversial as they involve in the destruction of human embryos (Lo & Parham, 2009). AFSCs are an ideal cell source, that provides easy access either during or after pregnancy where it can be obtained from small volumes (2-5 mL) of amniotic fluid, straight forward isolation and amplification of SCs, able to differentiate into lineages representative of all three germ layers, the potential to exercise immunomodulatory effects, and do not raise such intense ethical issues (Cananzi et al., 2009; Cananzi et al., 2011; Diaco et al., 2015; Tsai et al., 2004). Importantly, the advantages of AFSCs over ESCs are they do not form tumours when injected *in vivo* or in severe combined immunodeficient mice (Cananzi et al., 2009; Siegel et al., 2007).

1.2 Problem statement

Gangrenous wound are slow to heal and prone to complications such as infection. A series of multiple mechanisms, including decreased cell and growth factor response, lead to diminished peripheral blood flow and decreased local angiogenesis, all of which can contribute to lack of healing in hyperglycemic patients (Brem & Tomic-Canic, 2007). In spite of significant advances in medical and surgical wound care, treatment of chronic cutaneous wounds remains challenging. There are so many scientific and clinical ways in stimulating the wound repairs those involving drugs, plants, SCs etc. Nowadays, there is much scientific and clinical interest in the potential of SCs therapies to stimulate wound repairs. Studies have reported that the mechanism by which stem cells participate in tissue repair seems to be related to their awide range of growth factors, cytokines and chemokines that can be defined as stem cells secretome (Jayaraman et al., 2013). These molecules can be traced from the spent media or CM that harvested from cultured cells. Nowadays, CM has shown a successful outcome and serves as a new treatment modality in regenerative medicine and numerous cell based therapies including wound healing (Jayaraman et al., 2013; Walter et al., 2010). This gives support to the use of CM in the wound healing by modulating the wound repair without the present of SCs in the wound. Even so, the details of this method remain uncertain and need to be proved before taken as fact.

1.3 Justification of the study

Amniotic fluid stem cells (AFSCs) plays role in enhanced wound healing by secreting factors including growth factors, chemokines and cytokines (Chen et al., 2008; Jayaraman et al., 2013). These factors can be traced in the CM or spent medium harvested from cultured cells and serve as a tool among cells to communicate (Shohara et al., 2012). Most recently, CM has been used as a substitute for numerous cellular based therapies including wound healing in pre-clinical studies (Walter et al., 2010). Two major classes of regulators of cytokines and growth factors, and their effect on healing diabetic wounds are the focus of this study. Besides that, recent evidence had shown that CM from adipose-derived mesenchymal stem cells (AD-MSC), bone marrow-derived mesenchymal stem cells

(BM-MSC) and amniotic fluid-derived mesenchymal stem cells (AF-MSC) enhanced and accelerate the wound healing in both *in vitro* and *in vivo* (Jun et al., 2014). These studies claim that the CM also has the ability to promote the skin regeneration. This has encouraged the use of CM as alternative stem cells based therapy-conditioned medium derived growth factors and cytokines in wound healing by modulating wound repair without stem cells being present in the wound.

Rat amniotic fluid stem cells (rAFSCs) have been proven as pluripotent stems cells based on their ability to differentiate into three primary germ layers and the expression of OCT4, which is a marker for pluripotent cells (Mun-Fun et al., 2015). In pluripotent cells, OCT4 is highly expressed and rapidly decrease upon differentiation (Shi & Jin, 2010). The rAFSCs are expected to accelerate the wound healing process by stimulating the proliferation and migration of the diabetic human dermal fibroblast (HDF-D). The HDF-D will be used as a model to mimics diabetic wound healing *in vitro*. The rAFSCs are also expected to secret factors in CM that may enhance HDF-D migration to enhance wound closure. These research findings will be provided systematic scientific evidence for improving the wound healing. The evidence will provide an effective cost for the wound care that lessens the burden of the health systems and develop new product for wound healing. The projects have the potentials in generating revenue through commercialization of research products, licensing and royalties. The projects enhance the development of human capitals and experts, and the employment opportunities. The developed products may generate wealth in the health care industry.

1.4 General objective

The general aim of this study is to establish and characterise rat amniotic fluid stem cells-conditioned medium (rAFSCs-CM) that suitable for wound healing diabetic model.

1.5 Specific objectives

The specific objectives of this study are:

1) To develop rat amniotic fluid stem cells conditioned medium (rAFSCs-CM) treatment for *in vitro* diabetic wound healing model.

2) To determine the cytokines and growth factor secreted by rAFSCs in conditioned medium (CM).

3) To study the potential of the rAFSCs-CM secreted cytokines and growth factors that may have influence the Diabetic Human Dermal Fibroblast (HDF-D) cell proliferation and migrations.

4) To determine the most suitable CM between the rAFSCs-CM with presence of leukemia inhibitory factor (LIF+) or absence of LIF (LIF-).

1.6 Hypothesis

The hypothesis of the study is rAFSCs-CM secreted various growth factors that promote proliferation and migration of the HDF-D in *in vitro* diabetic wound healing model.

REFERENCES

Abdulrazzak, H., Moschidou, D., Jones, G., & Guillot, P. V. (2010). Biological characteristics of stem cells from foetal , cord blood and extraembryonic tissues. *Journal of The Royal Society Interface*, *7*, S689–S706.

Abranches, E., Bekman, E., & Henrique, D. (2013). Generation and characterization of a novel mouse embryonic stem cell line with a dynamic reporter of Nanog expression. *PloS One*, *8*(3), e59928.

Alonso, L., & Fuchs, E. (2003). Stem cells of the skin epithelium. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 11830–11835. http://doi.org/10.1073/pnas.1734203100

Ambady, S., Malcuit, C., Kashpur, O., Kole, D., Holmes, W. F., Hedblom, E., … Dominko, T. (2010). Expression of NANOG and NANOGP8 in a variety of undifferentiated and differentiated human cells. *Int J Dev Biol*, *54*(0), 1743–1754. http://doi.org/10.1387/ijdb.103192sa

Anum, S. Z., Muzavir, S. R., Hassan, A., Ali Khan, A., & Ahmad, A. (2015). Amniotic Fluid-Derived Stem Cells (AFSC) and Their Application in Cell Therapy and Tissue Engineering. *Razavi International Journal of Medicine*, *3*(1), 1–6. http://doi.org/10.5812/rijm.20135

Arnhold, S., Glüer, S., Hartmann, K., Raabe, O., Addicks, K., Wenisch, S., & Hoopmann, M. (2011). Amniotic-Fluid Stem Cells: Growth Dynamics and Differentiation Potential after a CD-117-Based Selection Procedure. *Stem Cells International*, *2011*, 715341. http://doi.org/10.4061/2011/715341

Baghaban Eslaminejad, M., & Jahangir, S. (2012). Amniotic fluid stem cells and their application in cell-based tissue regeneration. *International Journal of Fertility & Sterility*, *6*(3), 147–156.

Bai, J., Wang, Y., Liu, L., Jie, C., Yang, W., Lianru, G., & Wang, Y. (2012). Human amniotic fluid-derived c-kit + and c-kit 2 stem cells: growth characteristics and some differentiation potential capacities comparison. *Cytotechnology*, *64*, 577–589. http://doi.org/10.1007/s10616-012-9441-6

Bajek, A., Olkowska, J., Gurtowska, N., Kloskowski, T., Walentowicz-Sadlecka, M., Sadlecki, P., … Drewa, T. (2014). Human amniotic-fluid-derived stem cells: a unique source for regenerative medicine. *Expert Opinion on Biological Therapy*, *14*(6), 831–9.

Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H., & Tomic-Canic, M. (2008). Growth factors and cytokines in wound healing. *Wound Repair and Regeneration*, *16*, 585–601. http://doi.org/10.1111/j.1524-475X.2008.00410.x

Bates, D. O., Heald, R. I., Curry, F. E., & Williams, B. (2001). Vascular endothelial growth factor increases Rana vascular permeability and compliance by different

signalling pathways. *Journal of Physiology*, *533.1*, 263–272.

Becker, A. J., McCulloch, E. A., & Till, J. E. (1963). Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature*, *197*(4866), 452–454. http://doi.org/10.1038/197452a0

Bhang, S. H., Lee, S., Shin, J.-Y., Lee, T.-J., Jang, H.-K., & Kim, B.-S. (2014). Efficacious and Clinically Relevant Conditioned Medium of Human Adipose-derived Stem Cells for Therapeutic Angiogenesis. *Molecular Therapy*, *22*(4), 862–72. http://doi.org/10.1038/mt.2013.301

Bild, D. E., Selby, J. V, Sinnock, P., Browner, W. S., Braveman, P., & Showstack, J. A. (1989). Lower-extremity amputation in people with diabetes. Epidemiology and prevention. *Diabetes Care*, *12*(1), 24–31.

Blasi, A., Martino, C., Balducci, L., Saldarelli, M., Soleti, A., Navone, S. E., … Alessandri, G. (2011). Dermal fibroblasts display similar phenotypic and differentiation capacity to fat-derived mesenchymal stem cells, but differ in anti-inflammatory and angiogenic potential. *Vascular Cell*, *3*(1), 5. http://doi.org/10.1186/2045-824X-3-5

Blumberg, S. N., Berger, A., Hwang, L., Pastar, I., Warren, S. M., & Chen, W. (2012). The role of stem cells in the treatment of diabetic foot ulcers. *Diabetes Research and Clinical Practice*, *96*(1), 1–9. http://doi.org/10.1016/j.diabres.2011.10.032

Braiman-Wiksman, L., Solomonik, I., Spira, R., & Tennenbaum, T. (2007). Novel Insights into Wound Healing Sequence of Events. *Toxicologic Pathology*, *35*, 767–779. http://doi.org/10.1080/01926230701584189

Branski, L. K., Gauglitz, G. G., Herndon, D. N., & Jeschke, M. G. (2009). A review of gene and stem cell therapy in cutaneous wound healing. *Burns*, *35*(2), 171–180. http://doi.org/10.1016/j.burns.2008.03.009

Breitbart, A. S., Laser, J., Parrett, B., Porti, D., Grant, R. T., Grande, D. a, & Mason, J. M. (2003). Accelerated diabetic wound healing using cultured dermal fibroblasts retrovirally transduced with the platelet-derived growth factor B gene. *Annals of Plastic Surgery*, *51*(4), 409–414. http://doi.org/10.1097/01.SAP.0000084461.83554.71

Brem, H., & Tomic-Canic, M. (2007). Cellular and molecular basis of wound healing in diabetes. *The Journal of Clinical Investigation*, *117*(5), 1219–1222. http://doi.org/10.1172/JCI32169.Despite

Brown, M., & Wittwer, C. (2000). Flow cytometry: Principles and clinical applications in hematology. *Clinical Chemistry*, *46*(8 B), 1221–1229.

Buck, M., Houglum, K., & Chojkier, M. (1996). Tumor Necrosis Factor-a Inhibits Collagen al(I) Gene Expression and Wound Healing in a Murine Model of Cachexia. *American Journal of Pathology*, *149*(1), 195–204. Retrieved from

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1865213/pdf/amjpathol00031-0191.pdf

Cananzi, M., Atala, A., & Coppi, P. de. (2011). Stem Cells from Amniotic Fluid. *Principles of Regenerative Medicine*, 223–239. http://doi.org/10.1016/B978-0-12-381422-7.10012-4

Cananzi, M., Atala, A., & Coppi, P. De. (2009). Stem cells derived from amniotic fluid : new potentials in regenerative medicine. *Reproductive BioMedicine Online*, *18*(1), 17–27. http://doi.org/10.1016/S1472-6483(10)60111-3

Cananzi, M., & De Coppi, P. (2012). CD117 (+) amniotic fluid stem cells: state of the art and future perspectives. *Organogenesis*, *8*(3), 77–88. http://doi.org/10.4161/org.22426

Carraro, G., Perin, L., Sedrakyan, S., Giuliani, S., Tiozzo, C., Lee, J., … Warburton, D. (2008). Human Amniotic Fluid Stem Cells Can Integrate and Differentiate into Epithelial Lung Lineages. *Stem Cells*, *26*(11), 2902–2911. http://doi.org/10.1634/stemcells.2008-0090

Cavaleri, F., & Scholer, H. R. (2003). Nanog: A New Recruit to the Embryonic Stem Cell Orchestra. *Cell*, *113*, 551–557.

Cha, J., & Falanga, V. (2007). Stem cells in cutaneous wound healing. *Clinics in Dermatology*, *25*(1), 73–78. http://doi.org/10.1016/j.clindermatol.2006.10.002

Chambers, I., Colby, D., Robertson, M., Nichols, J., Lee, S., Tweedie, S., & Smith, A. (2003). Functional Expression Cloning of Nanog , a Pluripotency Sustaining Factor in Embryonic Stem Cells. *Cell*, *113*(5), 643–655.

Chen, J., Lu, Z., Cheng, D., Peng, S., & Wang, H. (2011). Isolation and Characterization of Porcine Amniotic Fluid- Derived Multipotent Stem Cells. *PLoS ONE*, *6*(5), e19964. http://doi.org/10.1371/journal.pone.0019964

Chen, L., Tredget, E. E., Wu, P. Y. G., Wu, Y., & Wu, Y. (2008). Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS ONE*, *3*(4). http://doi.org/10.1371/journal.pone.0001886

Chen, X., & Thibeault, S. L. (2011). Role of TNF–α in Wound Repair in Human Vocal Fold Fibroblasts. *Laryngoscope*, *120*(9), 1819–1825. http://doi.org/10.1002/lary.21037.Role

Choi, S. A., Choi, H. S., Kim, K. J., Lee, D. S., Lee, J. H., Park, J. Y., … Kim, M. K. (2013). Isolation of canine mesenchymal stem cells from amniotic fluid and differentiation into hepatocyte-like cells. *In Vitro Cellular and Developmental Biology - Animal*, *49*, 42–51. http://doi.org/10.1007/s11626-012-9569-x

Cooney, R., Iocono, J., Maish, G., Smith, J. S., & Ehrlich, P. (1997). Tumor necrosis factor mediates impaired wound healing in chronic abdominal sepsis. *The*

Journal of Trauma, *42*(3), 415–20. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9095108

Corral, C. J., Siddiqui, a, Wu, L., Farrell, C. L., Lyons, D., & Mustoe, T. a. (1999). Vascular endothelial growth factor is more important than basic fibroblastic growth factor during ischemic wound healing. *Archives of Surgery*, *134*(2), 200–205. http://doi.org/10.1001/archsurg.134.2.200

Da Sacco, S., Sedrakyan, S., Boldrin, F., Giuliani, S., Parnigotto, P., Habibian, R., … Perin, L. (2010). Human Amniotic Fluid as a Potential New Source of Organ Specific Precursor Cells for Future Regenerative Medicine Applications. *The Journal of Urology*, *183*(3), 1193–1200. http://doi.org/10.1016/j.juro.2009.11.006

Dasu, M. R., Devaraj, S., & Jialal, I. (2007). High glucose induces IL-1^{N_L} expression in human monocytes: mechanistic insights. *American Journal of Physiology - Endocrinology and Metabolism*, *293*, E337–E346. Retrieved from http://ajpendo.physiology.org/content/ajpendo/293/1/E337.full.pdf

De Coppi, P., Bartsch, G., Siddiqui, M. M., Xu, T., Santos, C. C., Perin, L., … Atala, A. (2007). Isolation of amniotic stem cell lines with potential for therapy. *Nature Biotechnology*, *25*(1), 100–6. http://doi.org/10.1038/nbt1274

Delo, D. M., De Coppi, P., Bartsch, G., & Atala, A. (2006). Amniotic Fluid and Placental Stem Cells. *Methods in Enzymology*, *419*(1948), 426–438. http://doi.org/10.1016/S0076-6879(06)19017-5

Diaco, N., Diamandis, Z., & Borlongan, C. (2015). Amniotic fluid-derived stem cells as an effective cell source for transplantation therapy in stroke. *Brain Circulation*, *1*(2), 119. http://doi.org/10.4103/2394-8108.172881

Di Santo, S., Yang, Z., Wyler von Ballmoos, M., Voelzmann, J., Diehm, N., Baumgartner, I., & Kalka, C. (2009). Novel Cell-Free Strategy for Therapeutic Angiogenesis: In Vitro Generated Conditioned Medium Can Replace Progenitor Cell Transplantation. *PLoS ONE*, *4*(5), e5643. http://doi.org/10.1371/journal.pone.0005643

Dinarello, C. A. (2004). Therapeutic strategies to reduce IL-1 activity in treating local and systemic inflammation. *Current Opinion in Pharmacology*, *4*, 378–385. Retrieved from http://ac.els-cdn.com/S1471489204000906/1-s2.0-S1471489204000906-main.pdf?_tid=1286342e-6a4d-11e7-8a15-00000aacb362&acdnat=1500226671_e1c00c0a809134dac95b705376a1b40e

Dinarello, C. A. (2009). Immunological and Inflammatory Functions of the Interleukin-1 Family. *Annual Review of Immunology*, *27*, 519–50. http://doi.org/10.1146/annurev.immunol.021908.132612

Donovan, P. J. (2001). High Oct-ane fuel powers the stem cell. *Nature Genetics*, *29*, 246–247. http://doi.org/10.1038/ng1101-246

Dvorak, H. F., Brown, L. F., Detmar, M., & Dvorak, A. M. (1995). Vascular

Permeability Factor/Vascular Endothelial Growth Factor, Microvascular Hyperpermeability, and Angiogenesis. *American Journal of Pathology*, *146*(5), 1029–1039. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1869291/pdf/amjpathol00053-0007.pdf

Enoch, S., & Leaper, D. J. (2007). Basic science of wound healing. *Surgery*, *26*(2), 31–37.

Esmaeelinejad, M., Bayat, M., Darbandi, H., Bayat, M., & Mosaffa, N. (2014). The effects of low-level laser irradiation on cellular viability and proliferation of human skin fibroblasts cultured in high glucose mediums. *Lasers in Medical Science*, *29*(1), 121–9. http://doi.org/10.1007/s10103-013-1289-2

Eve, David J.Phillip J. Marty, Robert J. McDermott, Stephen K. Klasko, and P. R. S. (2008). Stem Cell Research and Health Education. *American Journal of Health Education*, *39*(3), 167–179. http://doi.org/10.1016/j.cmet.2012.08.002.

Falanga, V. (2005). Wound healing and its impairment in the diabetic foot. *Lancet*, *366*, 1736–1743.

Fauza, D. (2004). Amniotic fluid and placental stem cells. *Best Practice & Research. Clinical Obstetrics & Gynaecology*, *18*(6), 877–91. http://doi.org/10.1016/j.bpobgyn.2004.07.001

Ferdaos, N., Karuppiah, T., Rosli, R., Yazid, M. N., & Nordin, N. (2011). Evaluation of Two Cell Culture Media in Culturing Rat Full Term Amniotic Fluid Cells. *Malaysian Journal of Medicine and Health Sciences*, *7*(2), 81–85.

Ferdaos, N., Nathan, S., & Nordin, N. (2008). Prospective full-term-derived pluripotent amniotic fluid stem (AFS) cells. *The Medical Journal of Malaysia*, *63 Suppl A*, 75–6.

Ferdaos, N., & Nordin, N. (2012). Human Amniotic Fluid Cells And Their Future Perspectives. *Regenerative Research*, *1*(2), 14–19.

Ferrara, N. (2004). Vascular Endothelial Growth Factor: Basic Science and Clinical Progress. *Endocrine Reviews*, *25*(4), 581–611. http://doi.org/10.1210/er.2003-0027

Ferrara, N., & Bunting, S. (1996). Vascular endothelial growth factor, a specific regulator of angiogenesis. *Current Opinion in Nephrology and Hypertension*, *5*(1), 35–44. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8834160

Ferrari, G., Cook, B. D., Terushkin, V., Pintucci, G., & Mignatti, P. (2009). Transforming growth factor-beta 1 (TGF-β1) induces angiogenesis through vascular endothelial growth factor (VEGF)-mediated apoptosis. *Journal of Cellular Physiology*, *219*(2), 449–458. http://doi.org/10.1002/jcp.21706

Fugger, L., Morling, N., Bendtzen, K., Ryder, L., Andersen, V., Heilman, C., …

Svejgaard, A. (1989). IL-6 gene polymorphism in rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, systemic lupus erythematosus, and in healthy Danes. *Journal of Immunogenetics*, *16*(6), 461–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2577114

Fukuoka, H., Suga, H., Narita, K., Watanabe, R., & Shintani, S. (2012). The Latest Advance in Hair Regeneration Therapy Using Proteins Secreted by Adipose-Derived Stem Cells. *American Journal of Cosmetic Surgery*, *29*(4), 273–282. http://doi.org/10.5992/AJCS-D-12-00015.1

Furue, M., Okamoto, T., Hayashi, Y., Okochi, H., Fujimoto, M., Myoishi, Y., … Sato, J. D. (2005). Leukemia Inhibitory Factor As An Anti-Apoptotic Mitogen For Pluripotent Mouse Embryonic Stem Cells In A Serum-Free Medium Without Feeder Cells. *In Vitro Cellular & Developmental Biology - Animal*, *41*, 19–28.

Galiano, R. D., Tepper, O. M., Pelo, C. R., Bhatt, K. A., Callaghan, M., Bastidas, N., … Gurtner, G. C. (2004). Topical Vascular Endothelial Growth Factor Accelerates Diabetic Wound Healing through Increased Angiogenesis and by Mobilizing and Recruiting Bone Marrow-Derived Cells. *American Journal of Pathology*, *164*(6), 1935–1947. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1615774/pdf/JPATH1640019 35.pdf

Gallucci, R. ., Sloan, D. ., Heck, J. ., Murray, A. ., & O'Dell, S. . (2004). Interleukin 6 indirectly induces keratinocyte migration. *Journal of Investigative Dermatology*, *122*(3), 764–72. Retrieved from http://ac.els-cdn.com/S0022202X15307004/1-s2.0-S0022202X15307004-main.pdf?_tid=d2882026-6a15-11e7-8107-00000aacb361&acdnat=1500202941_7db13291914f25db2b3edac45907f689

Gallucci, R. M., Simeonova, P. P., Matheson, J. M., Kommineni, C., Guriel, J. L., Sugawara, T., & Luster, M. I. (2000). Impaired cutaneous wound healing in interleukin-6-deficient and immunosuppressed mice. *The FASEB Journal*, *14*(15), 2525–2531. http://doi.org/10.1096/fj.00-0073com

Gao, L., Thilakavathy, K., & Nordin, N. (2013). A plethora of human pluripotent stem cells. *Cell Biology International*, *37*(9), 875–887. http://doi.org/10.1002/cbin.10120

Gao, L., Zhao, M., Ye, W., Huang, J., Chu, J., Yan, S., … Zeng, R. (2016). Inhibition of glycogen synthase kinase-3 (GSK3) promotes the neural differentiation of full-term amniotic fluid-derived stem cells towards neural progenitor cells. *Tissue and Cell*, *48*, 312–320. http://doi.org/10.1016/j.tice.2016.06.001

Gao, Y., Zhu, Z., Zhao, Y., Hua, J., Ma, Y., & Guan, W. (2014). Multilineage potential research of bovine amniotic fluid mesenchymal stem cells. *International Journal of Molecular Sciences*, *15*(3), 3698–3710. http://doi.org/10.3390/ijms15033698

Goel, S., Fujihara, M., Minami, N., Yamada, M., & Imai, H. (2008). Expression of

NANOG, but not POU5F1, points to the stem cell potential of primitive germ cells in neonatal pig testis. *Society for Reproduction and Fertility*, *135*, 785–795. http://doi.org/10.1530/REP-07-0476

Goodson, W. H., & Hunt, T. K. (1979). Wound healing and the diabetic patient. *Surgery, Gynecology & Obstetrics*, *149*(4), 600–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/483144

Goova, M. T., Li, J., Kislinger, T., Qu, W., Lu, Y., Bucciarelli, L. G., … Schmidt, A. M. (2001). Blockade of Receptor for Advanced Glycation End- Products Restores Effective Wound Healing in Diabetic Mice. *American Journal of Pathology*, *159*(2), 513–525. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1850533/pdf/2771.pdf

Goren, I., Kämpfer, H., Podda, M., Pfeilschifter, J., & Frank, S. (2003). Leptin and Wound Inflammation in Diabetic ob/ob Mice: Differential Regulation of Neutrophil and Macrophage Influx and a Potential Role for the Scab as a Sink for Inflammatory Cells and Mediators. *Diabetes*, *52*, 2821–2832. Retrieved from http://diabetes.diabetesjournals.org/content/diabetes/52/11/2821.full.pdf

Goren, I., Müller, E., Pfeilschifter, J., & Frank, S. (2006). Severely Impaired Insulin Signaling in Chronic Wounds of Diabetic ob/ob Mice. *The American Journal of Pathology*, *168*(3), 765–777. http://doi.org/10.2353/ajpath.2006.050293

Graf, U., Casanova, E. A., & Cinelli, P. (2011). The Role of the Leukemia Inhibitory Factor (LIF) — Pathway in Derivation and Maintenance of Murine Pluripotent Stem Cells. *Genes*, *2*, 280–297. http://doi.org/10.3390/genes2010280

Greenhalgh, D. G. (2003). Wound healing and diabetes mellitus. *Clinics in Plastic Surgery*, *30*, 37–45. http://doi.org/10.1016/S0094-1298(02)00066-4

Grossman, R. M., Krueger, J., Yourish, D., Granelli-Piperno, A., Murphy, D. P., May, L. T., … Gottlieb, A. B. (1989). Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. *Proceedings of the National Academy of Sciences*, *86*, 6367–6371. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC297840/pdf/pnas00283-0364.pdf

Guillot, P. V., Gotherstrom, C., Chan, J., Kurata, H., & Fisk, N. M. (2007). Human First-Trimester Fetal MSC Express Pluripotency Markers and Grow Faster and Have Longer Telomeres Than Adult MSC. *Stem Cells*, *25*, 646–654. http://doi.org/10.1634/stemcells.2006-0208

Gulcan, E., ßegul Kuçuk, A., Çayci, K., Tosun, M., Emre, H., Koral, L., … Avsar, U. (2012). Topical effects of nebivolol on wounds in diabetic rats. *European Journal of Pharmaceutical Sciences* , *47*, 451–455. http://doi.org/10.1016/j.ejps.2012.06.017

Guo, S., & DiPietrio, L. A. (2010). Factors Affecting Wound Healing. *Journal of Dental Research*, *89*(3), 219–229. http://doi.org/10.1177/0022034509359125

Hamazaki, T., Oka, M., Yamanaka, S., & Terada, S. (2004). Aggregation of embryonic stem cells induces Nanog repression and primitive endoderm differentiation. *Journal of Cell Science*, *117*(23), 5681–5686. http://doi.org/10.1242/jcs.01489

Hamid, A. A., Joharry, M. K., Mun-Fun, H., Hamzah, S. N., Rejali, Z., Yazid, M. N., … Nordin, N. (2017). Highly potent stem cells from full-term amniotic fluid: A realistic perspective. *Reproductive Biology*, *17*(1), 9–18. http://doi.org/10.1016/j.repbio.2017.02.001

Hamzah, S. N., Vidyadaran, S., & Nordin, N. (2014). Differentiation of Rat Full Term Amniotic Fluid Stem Cells Into Dopaminergic Neurons Phenotype Via Adherent and Non-Adherent. *Regenerative Research*, *3*(2), 140–141.

Han, W., Zhong-Ying, D., & Hua-Yan, W. (2009). Growth and identification of human amniotic fluid stem cells and analysis of their influencing factors. *Chinese Journal of Agricultural Biotechnology*, *6*(1), 75–79. http://doi.org/10.1017/S1479236209002630

Hart, A. H., Hartley, L., Ibrahim, M., & Robb, L. (2004). Identification, Cloning and Expression Analysis of the Pluripotency Promoting Nanog Genes in Mouse and Human. *Developmental Dynamics*, *230*, 187–198. http://doi.org/10.1002/dvdy.20034

Hatano, S., Tada, M., Kimura, H., Yamaguchi, S., Kono, T., Nakano, T., … Tada, T. (2005). Pluripotential competence of cells associated with Nanog activity. *Mechanisms of Development*, *122*, 67–69. Retrieved from http://ac.els-cdn.com/S0925477304002138/1-s2.0-S0925477304002138-main.pdf?_tid=fce745e0-60c4-11e7-ae1d-00000aacb360&acdnat=1499178663_7f1deeddf913523f2c1e23ae8dbb2468

He, Z., Li, J., Zhen, C., Feng, L., & Ding, X. (2006). Effect of leukemia inhibitory factor on embryonic stem cell differentiation : implications for supporting neuronal differentiation. *Acta Pharmacologica Sinica*, *27*(1), 80–90. http://doi.org/10.1111/j.1745-7254.2006.00254.x

Hehenberger, K., & Hansson, A. (1997). High glucose-induced growth factor resistance in human fibroblasts can be reversed by antioxidants and protein kinase C-inhibitors. *Cell Biochemistry and Function*, *15*(3), 197–201. http://doi.org/10.1002/(SICI)1099-0844(199709)15:3<197::AID-CBF740>3.0.CO;2-7

Hemberger, M., Yang, W., Natale, D., Brown, T. L., Dunk, C., Gargett, C. E., & Tanaka, S. (2008). Stem Cells from Fetal Membranes - A Workshop Report. *Placenta 29, Supplement A, Trophoblast Research*, *22*, 17–19. http://doi.org/10.1016/j.placenta.2007.11.006

Hima Bindu, A., & Srilatha, B. (2011). Stem Cell Potency of Various Types of Stem Cells and their Transplantation. *Journal of Stem Cell Research & Therapy*, *1*(3),

115. Retrieved from http://dx.doi.org/10.4172/2157-7633.1000115

Ho, J. C. Y., Lai, W.-H., Li, M.-F., Au, K.-W., Yip, M.-C., Wong, N. L. Y., … Tse, H.-F. (2012). Reversal of endothelial progenitor cell dysfunction in patients with type 2 diabetes using a conditioned medium of human embryonic stem cell-derived endothelial cells. *Diabetes/Metabolism Research And Reviews Research*, *28*(5), 462–473. http://doi.org/10.1002/dmrr

Hoehn, H., Bryant, E. M., Karp, L. E., & Martin, G. M. (1974). Cultivated Cells from Diagnostic Amniocentesis in Second Trimester Pregnancies. I. Clonal Morphology and Growth Potential. *Pediatric Research*, *8*(8), 746–754. http://doi.org/10.1203/00006450-197408000-00003

Hoehn, H., Bryant, E. M., Karp, L. E., & Martin, G. M. (1975). Cultivated cells from diagnostic amniocentesis in second trimester pregnancies. II. Cytogenetic parameters as functions of clonal type and preparative technique. *Clinical Genetics*, *7*(1), 29–36. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1167818

Hoffman, M., & Monroe, D. M. (2010). Wound healing in haemophilia-breaking the vicious cycle. *Haemophilia*, *16*(3), 13–18. http://doi.org/10.1111/j.1365-2516.2010.02254.x

Honnegowda, T. M., Kumar, P., Udupa, E. P., Kumar, S., Kumar, U., & Rao, P. (2015). Role of angiogenesis and angiogenic factors in acute and chronic wound healing. *Plastic and Aesthetic Research*, *2*(5), 243–249. http://doi.org/10.4103/2347-9264.165438

Howdieshell, T. R., Callaway, D., Webb, W. L., Gaines, M. D., Procter, C. D., Sathyanarayana, … McNeil, P. L. (2001). Antibody neutralization of vascular endothelial growth factor inhibits wound granulation tissue formation. *Journal of Surgical Research* , *96*(2), 173–182. Retrieved from http://ac.els-cdn.com/S0022480401960895/1-s2.0-S0022480401960895-main.pdf?_tid=fb255a82-6a04-11e7-9a91-00000aacb361&acdnat=1500195708_c94f3bc388a71990e5fbd86e07ffc0c6

Hu, L., Zhao, J., Liu, J., Gong, N., & Chen, L. (2013). Effects of adipose stem cell-conditioned medium on the migration of vascular endothelial cells, fibroblasts and keratinocytes. *Experimental and Therapeutic Medicine*, *5*(3), 701–706. http://doi.org/10.3892/etm.2013.887

Huh, M.-I., Kim, M.-S., Kim, H.-K., & Lim, J. O. (2014). Effect of conditioned media collected from human amniotic fluid-derived stem cells (hAFSCs) on skin regeneration and photo-aging. *Tissue Engineering and Regenerative Medicine*, *11*(2), 171–177. http://doi.org/10.1007/s13770-014-0412-1

Hyun, I. (2010). The bioethics of stem cell research and therapy. *The Journal of Clinical Investigation*, *120*(1), 71–75. http://doi.org/10.1172/JCI40435

Iacono, E., Brunori, L., Pirrone, A., Pagliaro, P. P., Ricci, F., Tazzari, P. L., & Merlo,

B. (2012). Isolation, characterization and differentiation of mesenchymal stem cells from amniotic fluid, umbilical cord blood and Wharton's jelly in the horse. *Reproduction*, *143*, 455–468. http://doi.org/10.1530/REP-10-0408

Imanishi, J., Kamiyama, K., Iguchi, I., Kita, M., Sotozono, C., & Kinoshita, S. (2000). Growth Factors: Importance in Wound Healing and Maintenance of Transparency of the Cornea. *Progress in Retinal and Eye Research*, *19*(1), 113–129.

Jayaraman, P., Nathan, P., Vasanthan, P., Musa, S., & Govindasamy, V. (2013). Stem cells conditioned medium: A new approach to skin wound healing management. *Cell Biology International*, *37*, 1122–1128. http://doi.org/10.1002/cbin.10138

Jinesh, G. G., Chunduru, S., & Kamat, A. M. (2012). Smac mimetic enables the anticancer action of BCG-stimulated neutrophils through TNF- but not through TRAIL and FasL. *Journal of Leukocyte Biology*, *92*, 233–244. http://doi.org/10.1189/jlb.1211623

Johnson, K. E., & Wilgus, T. A. (2014). Vascular Endothelial Growth Factor and Angiogenesis in the Regulation of Cutaneous Wound Repair. *Advances In Wound Care*, *3*(10), 647–661. http://doi.org/10.1089/wound.2013.0517

Joo, S., Ko, I. K., Atala, A., Yoo, J. J., & Lee, S. J. (2012). Amniotic fluid-derived stem cells in regenerative medicine research. *Archives of Pharmacal Research*, *35*(2), 271–280. http://doi.org/10.1007/s12272-012-0207-7

Jun, E. K., Zhang, Q., Yoon, B. S., Moon, J.-H., Lee, G., Park, G., … You, S. (2014). Hypoxic conditioned medium from human amniotic fluid-derived mesenchymal stem cells accelerates skin wound healing through TGF-β/SMAD2 and PI3K/Akt pathways. *International Journal of Molecular Sciences*, *15*(1), 605–28. http://doi.org/10.3390/ijms15010605

Jung, J. Y., Shim, J. H., Choi, H., Lee, T. R., & Shin, D. W. (2015). Human dermal stem/progenitor cell-derived conditioned medium improves senescent human dermal fibroblasts. *International Journal of Molecular Sciences*, *16*(8), 19027–19039. http://doi.org/10.3390/ijms160819027

Karlmark, K. R., Freilinger, A., Marton, E., Rosner, M., Lubec, G., & Hengstschläger, M. (2005). Activation of ectopic Oct-4 and Rex-1 promoters in human amniotic fluid cells. *International Journal of Molecular Medicine.*, *16*, 987–992.

Kataoka, K., Medina, R. J., Kageyama, T., Miyazaki, M., Yoshino, T., Makino, T., & Huh, N.-H. (2003). Participation of adult mouse bone marrow cells in reconstitution of skin. *The American Journal of Pathology*, *163*(4), 1227–31. http://doi.org/10.1016/S0002-9440(10)63482-7

Kim, H. O., Choi, S. M., & Kim, H. S. (2013). Mesenchymal stem cell-derived secretome and microvesicles as a cell-free therapeutics for neurodegenerative

disorders. *Tissue Engineering and Regenerative Medicine*, *10*(3), 93–101. http://doi.org/10.1007/s13770-013-0010-7

Kim, J., Lee, J. H., Yeo, S. M., Chung, H. M., & Chae, J.-I. (2014). Stem cell recruitment factors secreted from cord blood-derived stem cells that are not secreted from mature endothelial cells enhance wound healing. *In Vitro Cellular and Developmental Biology - Animal*, *50*, 146–154. http://doi.org/10.1007/s11626-013-9687-0

Kim, J., Lee, Y., Kim, H., Hwang, K. J., Kwon, H. C., Kim, S. K., … You, J. (2007a). Human amniotic fluid-derived stem cells have characteristics of multipotent stem cells. *Cell Proliferation*, *40*(1), 75–90. http://doi.org/10.1111/j.1365-2184.2007.00414.x

Kim, S. W., Zhang, H. Z., Guo, L., Kim, J. M., & Kim, M. H. (2012). Amniotic mesenchymal stem cells enhance wound healing in diabetic NOD/SCID mice through high angiogenic and engraftment capabilities. *PLoS ONE*, *7*(7), e41105. http://doi.org/10.1371/journal.pone.0041105

Kim, W.-S., Park, B.-S., Sung, J.-H., Yang, J.-M., Park, S.-B., Kwak, S.-J., & Park, J.-S. (2007b). Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. *Journal of Dermatological Science*, *48*(1), 15–24. http://doi.org/10.1016/j.jdermsci.2007.05.018

Klemmt, P. (2012). Application of amniotic fluid stem cells in basic science and tissue regeneration. *Organogenesis*, *8*(3), 76–76.

Klemmt, P. A., Vafaizadeh, V., & Groner, B. (2011). The potential of amniotic fluid stem cells for cellular therapy and tissue engineering. *Expert Opinion on Biological Therapy*, *11*(10), 1297–1314. http://doi.org/10.1517/14712598.2011.587800

Klimanskaya, I., Rosenthal, N., & Lanza, R. (2008). Derive and conquer: sourcing and differentiating stem cells for therapeutic applications. *Nature Reviews Drug Discovery*, *7*(2), 131–142. http://doi.org/10.1038/nrd2403

Koch, A. E., Kronfeld-Harrington, L. B., Szekanecz, Z., Cho, M. M., Haines, G. K., Harlow, L. A., … Barr, W. G. (1993). In situ expression of cytokines and cellular adhesion molecules in the skin of patients with systemic sclerosis. Their role in early and late disease. *Pathobiology*, *61*(5–6), 239–46. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7507681

Koenen, T. B., Stienstra, R., Van Tits, L. J., De Graaf, J., Stalenhoef, A. F. H., Joosten, L. A. B., … Netea, M. G. (2011). Hyperglycemia Activates Caspase-1 and TXNIP-Mediated IL-1b Transcription in Human Adipose Tissue. *Diabetes*, *60*, 517–524. http://doi.org/10.2337/db10-0266

Lerman, O. Z., Galiano, R. D., Armour, M., Levine, J. P., & Gurtner, G. C. (2003). Cellular Dysfunction in the Diabetic Fibroblast Impairment in Migration, Vascular Endothelial Growth Factor Production, and Response to Hypoxia.

American Journal of Pathology, *162*(1), 303–312. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1851127/pdf/3458.pdf

Li, H., Fu, X., Ouyang, Y., Cai, C., Wang, J., & Sun, T. (2006). Adult bone-marrow-derived mesenchymal stem cells contribute to wound healing of skin appendages. *Cell and Tissue Research*, *326*(3), 725–736. http://doi.org/10.1007/s00441-006-0270-9

Li, W., Fan, J., Chen, M., Guan, S., Sawcer, D., Bokoch, G. M., & Woodley, D. T. (2004). Mechanism of human dermal fibroblast migration driven by type I collagen and platelet-derived growth factor-BB. *Molecular Biology of the Cell*, *15*(1), 294–309. http://doi.org/10.1091/mbc.E03-05-0352

Li, Y., Fan, J., Chen, M., Li, W., & Woodley, D. T. (2006). Transforming Growth Factor-Alpha: A Major Human Serum Factor that Promotes Human Keratinocyte Migration. *Journal of Investigative Dermatology*, *126*, 2096–2105. http://doi.org/10.1038/sj.jid.5700350

Lin, T. W., Cardenas, L., Glaser, D. L., & Soslowsky, L. J. (2006). Tendon healing in interleukin-4 and interleukin-6 knockout mice. *Journal of Biomechanics*, *39*, 61–69. http://doi.org/10.1016/j.jbiomech.2004.11.009

Lin, Z.-Q., Kondo, T., Ishida, Y., Takayasu, T., & Mukaida, N. (2003). Essential involvement of IL-6 in the skin wound-healing process as evidenced by delayed wound healing in IL-6-deficient mice. *Journal of Leukocyte Biology*, *73*(6), 713–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12773503

Lo, B., & Parham, L. (2009). Ethical Issues in Stem Cell Research. *Endocrine Reviews*, *30*(3), 204–213. http://doi.org/10.1210/er.2008-0031

Loukogeorgakis, S. P., & Coppi, P. De. (2016). Stem cells from amniotic fluid-Potential for regenerative medicine. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *31*, 45–57. http://doi.org/10.1016/j.bpobgyn.2015.08.009

Luckett, L. R., & Gallucci, R. M. (2007). Interleukin-6 (IL-6) modulates migration and matrix metalloproteinase function in dermal fibroblasts from IL-6KO mice. *British Journal of Dermatology*, *156*, 1163–1171. http://doi.org/10.1111/j.1365-2133.2007.07867.x

Mahmood, T., & Yang, P. (2012). Western Blot : Technique , Theory , and Trouble Shooting, *4*(9). http://doi.org/10.4103/1947-2714.100998

Martello, G., Bertone, P., & Smith, A. (2013). Identification of the missing pluripotency mediator downstream of leukaemia inhibitory factor. *The EMBO Journal*, *32*(19), 2561–74. http://doi.org/10.1038/emboj.2013.177

Mast, B. A., & Schultz, G. S. (1996). Interactions of cytokines, growth factors, and proteases in acute and chronic wounds. *Wound Repair and Regeneration*, *4*(4), 411–420. http://doi.org/10.1046/j.1524-475X.1996.40404.x

Mendes, J. J., Leandro, C. I., Bonaparte, D. P., & Pinto, A. L. (2012). A rat model of diabetic wound infection for the evaluation of topical antimicrobial therapies. *Comparative Medicine*, *62*(1), 37–48.

Merrill, B. J. (2012). Wnt Pathway Regulation of Embryonic Stem Cell Self-Renewal. *Cold Spring Harbor Laboratory Press*, *4*, a007971. http://doi.org/10.1101/cshperspect.a007971

Michel, P. E., Crettaz, D., Morier, P., Heller, M., Gallot, D., Tissot, J. D., … Rossier, J. S. (2006). Proteome analysis of human plasma and amniotic fluid by Off-Gel^{TM} isoelectric focusing followed by nano-LC-MS/MS. *Electrophoresis*, *27*(5–6), 1169–1181. http://doi.org/10.1002/elps.200500680

Mirza, R. E., Fang, M. M., Ennis, W. J., & Kohl, T. J. (2013). Blocking interleukin-1β induces a healing-associated wound macrophage phenotype and improves healing in type 2 diabetes. *Diabetes*, *62*(7), 2579–2587. http://doi.org/10.2337/db12-1450

Mirza, R., & Koh, T. J. (2011). Dysregulation of monocyte/macrophage phenotype in wounds of diabetic mice. *Cytokine*, *56*, 256–264. http://doi.org/10.1016/j.cyto.2011.06.016

Mitsui, K., Tokuzawa, Y., Itoh, H., Segawa, K., Murakami, M., Takahashi, K., … Yamanaka, S. (2003). The Homeoprotein Nanog Is Required for Maintenance of Pluripotency in Mouse Epiblast and ES Cells. *Cell*, *113*(5), 631–642. Retrieved from http://ac.els-cdn.com/S0092867403003933/1-s2.0-S0092867403003933-main.pdf?_tid=ef4f509a-60c8-11e7-8b44-00000aacb360&acdnat=1499180359_cb3bc1d88450d30335a91b5f00100baa

Moon, J.-H., Kwak, S. S., Park, G., Jung, H.-Y., Yoon, B. S., Park, J., … You, S. (2008). Isolation and characterization of multipotent human keloid-derived mesenchymal-like stem cells. *Stem Cells and Development*, *17*(4), 713–24. http://doi.org/10.1089/scd.2007.0210

Moser, R., Schleiffenbaum, B., Groscurth, P., & Fehr, J. (1989). Interleukin 1 and Tumor Necrosis Factor Stimulate Human Vascular Endothelial Cells to Promote Transendothelial Neutrophil Passage. *The Journal of Clinical Investigation*, *83*(2), 444–55. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC303700/pdf/jcinvest00083-0096.pdf

Mun-Fun, H., Ferdaos, N., Hamzah, S. N., Ridzuan, N., Hishama, N. A., Abdullah, S., … Nordin, N. (2015). Rat full term amniotic fluid harbors highly potent stem cells. *Research in Veterinary Science Journal*, *102*, 89–99. http://doi.org/10.1016/j.rvsc.2015.07.010

Naguib, G., Al-Mashat, H., Desta, T., & Graves, D. T. (2004). Diabetes Prolongs the Inflammatory Response to a Bacterial Stimulus Through Cytokine Dysregulation. *Journal of Investigative Dermatology*, *123*(1), 87–92.

http://doi.org/10.1111/J.0022-202X.2004.22711.X

Nichols, J., Evans, E. P., & Smith, A. G. (1990). Establishment of germ-line-competent embryonic stem (ES) cells using differentiation inhibiting activity. *Development*, *110*, 1341–1348. Retrieved from http://dev.biologists.org/content/develop/110/4/1341.full.pdf

Niwa, H., Burdon, T., Chambers, I., & Smith, A. (1998). Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. *Genes & Development*, *12*(13), 2048–60. http://doi.org/10.1101/gad.12.13.2048

Niwa, H., Miyazaki, J., & Smith, A. G. (2000). Quantitative expression of Oct-3 / 4 defines differentiation , dedifferentiation or self-renewal of ES cells. *Nature Genetics*, *24*, 372–376.

Nizard, J. (2010). Amniocentesis: technique and education. *Current Opinion in Obstetrics & Gynecology*, *22*, 152–154. http://doi.org/10.1097/GCO.0b013e32833723a0

Noronha, I. L., Cavaglieri, R. C., Janz, F. L., Duarte, S. A., Lopes, M. A. B., Zugaib, M., & Bydlowski, S. P. (2011). The potential use of stem cells derived from human amniotic fluid in renal diseases. *Kidney International Supplements*, *1*, 77–82. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4089735/pdf/kisup201118a.pdf

Odorico, J. S., Kaufman, D. S., & Thomson, J. A. (2001). Multilineage Differentiation from Human Embryonic Stem Cell Lines. *Stem Cells*, *19*, 193–204. http://doi.org/10.1634/stemcells.19-3-193

Pappa, K. I., & Anagnou, N. P. (2009). Novel sources of fetal stem cells : where do they fit on the developmental continuum? *Regenerative Medicine*, *4*(3), 423–433.

Paquet, P., & Pierard, G. E. (1996). lnterleukin-6 and the Skin Cutaneous diseases Cytokine Drugs Interleukin-6 Skin. *International Archives of Allergy and Immunology*, *109*, 308–317. Retrieved from https://www.karger.com/Article/Pdf/237257

Park, B.-S., Kim, W.-S., Choi, J.-S., Kim, H.-K., Won, J.-H., Ohkubo, F., & Fukuoka, H. (2010). Hair growth stimulated by conditioned medium of adipose-derived stem cells is enhanced by hypoxia: evidence of increased growth factor secretion. *Biomedical Research*, *31*(1), 27–34. Retrieved from https://www.jstage.jst.go.jp/article/biomedres/31/1/31_1_27/_pdf

Park, S.-J., Yoon, W.-G., Song, J.-S., Jung, H. S., Kim, C. J., Oh, S. Y., … Nirasawa, T. (2006). Proteome analysis of human amnion and amniotic fluid by two-dimensional electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Proteomics*, *6*(1), 349–363. http://doi.org/10.1002/pmic.200500084

Pawitan, J. A. (2014). Prospect of Stem Cell Conditioned Medium in Regenerative

Medicine. *BioMed Research International*, 1–14. http://doi.org/10.1155/2014/965849

Penn, J. W., Grobbelaar, A. O., & Rolfe, K. J. (2012). The role of the TGF-β family in wound healing, burns and scarring: a review. *International Journal of Burns and Trauma*, *2*(1), 18–28. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3415964&tool=pmcentrez&rendertype=abstract

Pesce, M., & Schöler, H. R. (2001). Oct-4: Gatekeeper in the Beginnings of Mammalian Development. *Stem Cells*, *19*, 271–278. http://doi.org/10.1634/stemcells.2004-0114

Phermthai, T., Odglun, Y., Julavijitphong, S., Titapant, V., Chuenwattana, P., Vantanasiri, C., & Pattanapanyasat, K. (2010). A novel method to derive amniotic fluid stem cells for therapeutic purposes. *BMC Cell Biology*, *11*(1), 79. http://doi.org/10.1186/1471-2121-11-79

Postlethwaite, A. E., Jorma Keski-Oja, N., Moses, H. L., Kang, A. H., Keski-Oja, J., Moses, H. L., & Kang, A. H. (1987). Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor beta. *The Journal of Experimental Medicine*, *165*(1), 251–256. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2188256/pdf/je1651251.pdf

Pratheesh, M. D., Gade, N. E., Nath Katiyar, A., Dubey, P. K., Sharma, B., Saikumar, G., … Sharma. (2013). Isolation, culture and characterization of caprine mesenchymal stem cells derived from amniotic fluid. *Research in Veterinary Science*, *94*, 313–319. http://doi.org/10.1016/j.rvsc.2012.08.002

Primo, L., Seano, G., Roca, C., Maione, F., Gagliardi, P. A., Sessa, R., … Bussolino, F. (2010). Increased Expression of α6 Integrin in Endothelial Cells Unveils a Proangiogenic Role for Basement Membrane. *Cancer Research*, *70*(14), 5759–5769. Retrieved from http://cancerres.aacrjournals.org/content/70/14/5759.full-text.pdf

Prusa, A.-R., Marton, E., Rosner, M., Bernaschek, G., & Hengstschläger, M. (2003). Oct-4-expressing cells in human amniotic fluid: a new source for stem cell research? *Human Reproduction (Oxford, England)*, *18*(7), 1489–1493. http://doi.org/10.1093/humrep/deg279

Pushparani, D. . (2015). Role of Cytokines in Periodontal Wound Healing Process -A Review. *Pharmaceutical Analytical Chemistry Open Access*, *1*(1), 106. http://doi.org/10.4172/2471-2698.1000106

Rao, X., Zhong, J., Zhang, S., Zhang, Y., Yu, Q., Yang, P., … Wang, C.-Y. (2011). Loss of Methyl-CpG–Binding Domain Protein 2 Enhances Endothelial Angiogenesis and Protects Mice Against Hind-Limb Ischemic Injury. *Circulation*, *123*(25), 2964–2974. http://doi.org/10.1161/CIRCULATIONAHA.110.966408

Rennie, K., Gruslin, A., Hengstschläger, M., Pei, D., Cai, J., Nikaido, T., & Bani-

Yaghoub, M. (2012). Applications of amniotic membrane and fluid in stem cell biology and regenerative medicine. *Stem Cells International*, *2012*, 1–13. http://doi.org/10.1155/2012/721538

Rodda, D. J., Chew, J., Loh, Y., Wang, B., Ng, H., & Robson, P. (2005). Transcriptional Regulation of Nanog by OCT4 and SOX2. *The Journal Of Biological Chemistry*, *280*(26), 24731–24737. http://doi.org/10.1074/jbc.M502573200

Rossi, B., Merlo, B., Colleoni, S., Iacono, E., Tazzari, P. L., Ricci, F., … Galli, C. (2014). Isolation and in Vitro Characterization of Bovine Amniotic Fluid Derived Stem Cells at Different Trimesters of Pregnancy. *Stem Cell Reviews and Reports*, *10*, 712–724. http://doi.org/10.1007/s12015-014-9525-0

Rota, C., Imberti, B., Pozzobon, M., Piccoli, M., De Coppi, P., Atala, A., … Morigi, M. (2012). Human amniotic fluid stem cell preconditioning improves their regenerative potential. *Stem Cells and Development*, *21*(11), 1911–23. http://doi.org/10.1089/scd.2011.0333

Roubelakis, M. G., Trohatou, O., & Anagnou, N. P. (2012). Amniotic fluid and amniotic membrane stem cells: marker discovery. *Stem Cells International*, *2012*, 107836. http://doi.org/10.1155/2012/107836

Rumalla, V. K., & Borah, G. L. (2001). Cytokines, Growth Factors, and Plastic Surgery. *Plastic And Reconstructive Surgery*, *108*(3), 719–733. Retrieved from http://www.lipteh.com/Study-Notes/General/Wound healing/Articles/cytokines.pdf

Sadiq, T. S., & Gerber, D. A. (2004). Stem cells in modern medicine: Reality or myth? *Journal of Surgical Research*, *122*(2), 280–291. http://doi.org/10.1016/j.jss.2004.04.025

Sato, M., Sawamura, D., Ina, S., Yaguchi, T., Hanada, K., & Hashimoto, I. (1999). In vivo introduction of the interleukin 6 gene into human keratinocytes: induction of epidermal proliferation by the fully spliced form of interleukin 6, but not by the alternatively spliced form. *Archives of Dermatological Research*, *291*(7–8), 400–404. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10482009

Sawamura, D., Meng, X., Ina, S., Sato, M., Tamai, K., Hanada, K., & Hashimoto, I. (1998). Induction of Keratinocyte Proliferation and Lymphocytic Infiltration by In Vivo Introduction of the IL-6 Gene into Keratinocytes and Possibility of Keratinocyte Gene Therapy for Inflammatory Skin Diseases Using IL-6 Mutant Genes. *The Journal of Immunology*, *161*(10), 5633–5639; Retrieved from http://www.jimmunol.org/content/jimmunol/161/10/5633.full.pdf

Sehgal, P. B. (1990). Interleukin-6: molecular pathophysiology. *The Journal of Investigative Dermatology*, *94*, 2S–6S. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2191052

Serakinci, N., & Keith, W. N. (2006). Therapeutic potential of adult stem cells.

European Journal of Cancer, *42*(9), 1243–1246. http://doi.org/10.1016/j.ejca.2006.01.036

Shah, J. M. Y., Omar, E., Pai, D. R., & Sood, S. (2012). Cellular events and biomarkers of wound healing. *Indian Journal of Plastic Surgery : Official Publication of the Association of Plastic Surgeons of India*, *45*(2), 220–228. http://doi.org/10.4103/0970-0358.101282

Shi, G., & Jin, Y. (2010). Role of Oct4 in maintaining and regaining stem cell pluripotency. *Stem Cell Research & Therapy*, *1*(5), 39. http://doi.org/10.1186/scrt39

Shin, L., & Peterson, D. A. (2012). Impaired Therapeutic Capacity of Autologous Stem Cells in a Model of Type 2 Diabetes. *Stem Cells Translational Medicine*, *1*, 125–135.

Shohara, R., Yamamoto, A., Takikawa, S., Iwase, A., Hibi, H., Kikkawa, F., & Ueda, M. (2012). Mesenchymal stromal cells of human umbilical cord Wharton's jelly accelerate wound healing by paracrine mechanisms. *Cytotherapy*, *14*(10), 1171–1181. http://doi.org/10.3109/14653249.2012.706705

Siegel, N., Rosner, M., Hanneder, M., Valli, A., & Hengstschläger, M. (2007). Stem cells in amniotic fluid as new tools to study human genetic diseases. *Stem Cell Reviews*, *3*(4), 256–64. http://doi.org/10.1007/s12015-007-9003-z

Siminovitch, L., McCulloch, E. A., & Till, J. E. (1963). the Distribution of Colony-Forming Cells Among Spleen Colonies. *Journal of Cell Physiology*, *62*(3), 327–336.

Singer, A. J., & Clark, R. A. F. (1999). Cutaneous Wound Healing. *The New England Journal of Medicine Review*, *341*(10), 738–746.

Singh, A. M., Hamazaki, T., Hankowski, K. E., & Terada, N. (2007). A heterogeneous expression pattern for Nanog in embryonic stem cells. *Stem Cells*, *25*(10), 2534–2542. http://doi.org/10.1634/stemcells.2007-0126

Skardal, A., Mack, D., Kapetanovic, E., Atala, A., Jackson, J. D., Yoo, J., & Soker, S. (2012). Bioprinted Amniotic Fluid-Derived Stem Cells Accelerate Healing of Large Skin Wounds. *Stem Cells Translational Medicine*, *1*(11), 792–802. http://doi.org/10.5966/sctm.2012-0088

Smith, A. G. (2001). Embryo-Derived Stem Cells : Of Mice And Men. *Annual Review of Cell and Developmental Biology*, *17*, 435–62. http://doi.org/10.1146/annurev.cellbio.17.1.435

Smith, A. G., Nichols, J., Robertson, M., & Rathjen, P. D. (1992). Differentiation inhibiting activity (DIA/LIF) and mouse development. *Developmental Biology*, *151*, 339–351. http://doi.org/0012-1606(92)90174-F [pii]

Smith AG, Heath JK, Donaldson DD, Wong GG, Moreau J, Stahl M, R. D. (1988).

Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. *Nature*, *336*, 688–690. Retrieved from https://www.nature.com/nature/journal/v336/n6200/pdf/336688a0.pdf

Steed, D. L. (1997). The role of growth factors in wound healing. *The Surgical Clinics of North America*, *77*(3), 575–86. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9194881

Sun, Q., Li, F., Li, H., Chen, R.-H., Gu, Y.-Z., Chen, Y., … Zhang, X.-G. (2015). Amniotic fluid stem cells provide considerable advantages in epidermal regeneration: B7H4 creates a moderate inflammation microenvironment to promote wound repair. *Scientific Reports*, *5*(October 2014), 11560. http://doi.org/10.1038/srep11560

Tam, J. C. W., Lau, K. M., Liu, C. L., To, M. H., Kwok, H. F., Lai, K. K., … Lau, C. B. S. (2011). The in vivo and in vitro diabetic wound healing effects of a 2-herb formula and its mechanisms of action. *Journal of Ethnopharmacology*, *134*(3), 831–838. http://doi.org/10.1016/j.jep.2011.01.032

Teo, A. K. K., & Vallier, L. (2010). Emerging use of stem cells in regenerative medicine. *The Biochemical Journal*, *428*(1), 11–23. http://doi.org/10.1042/BJ20100102

Teodelinda, M., Michele, C., Sebastiano, C., Ranieri, C., & Chiara, G. (2011). Amniotic liquid derived stem cells as reservoir of secreted angiogenic factors capable of stimulating neo-arteriogenesis in an ischemic model. *Biomaterials*, *32*, 3689–3699. http://doi.org/10.1016/j.biomaterials.2011.01.071

Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., & Jones, J. M. (1998). Embryonic Stem Cell Lines Derived from Human Blastocysts. *Science*, *282*, 1145–1147. Retrieved from http://science.sciencemag.org/content/sci/282/5391/1145.full.pdf

Till, J. E., & McCulloch, E. A. (1961). A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiation Research*, *14*(2), 213–222. http://doi.org/10.2307/3570892

Trengove, N. J., Bielefeldt-Ohmann, H., & Stacey, M. C. (2000). Mitogenic activity and cytokine levels in non-healing and healing chronic leg ulcers. *Wound Repair and Regeneration*, *8*(1), 13–25. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10760211

Trengove, N. J., Langton, S. R., & Stacey, M. C. (1996). Biochemical analysis of wound fluid from nonhealing and healing chronic leg ulcers. *Wound Repair and Regeneration*, *4*(2), 234–239. http://doi.org/10.1046/j.1524-475X.1996.40211.x

Trounson, A. (2007). A fluid means of stem cell generation. *Nature Biotechnology*, *25*, 62–63. http://doi.org/10.1038/nbt0107-62

Tsai, M.-S., Hwang, S.-M., Tsai, Y.-L., Cheng, F.-C., Lee, J.-L., & Chang, Y.-J. (2006). Clonal amniotic fluid-derived stem cells express characteristics of both

mesenchymal and neural stem cells. *Biology of Reproduction*, *74*(3), 545–51. http://doi.org/10.1095/biolreprod.105.046029

Tsai, M.-S., Lee, J.-L. L., Chang, Y.-J. J., & Hwang, S.-M. M. (2004). Isolation of human multipotent mesenchymal stem cells from second-trimester amniotic fluid using a novel two-stage culture protocol. *Human Reproduction*, *19*(6), 1450–1456. http://doi.org/10.1093/humrep/deh279

Tsigkos, S., Koutsilieris, M., & Papapetropoulos, A. (2003). Angiopoietins in angiogenesis and beyond. *Expert Opinion on Investigational Drugs*, *12*(6), 933–941. http://doi.org/10.1517/13543784.12.6.933

Tsourdi, E., Barthel, A., Rietzsch, H., Reichel, A., & Bornstein, S. R. (2013). Current aspects in the pathophysiology and treatment of chronic wounds in diabetes mellitus. *BioMed Research International*, *2013*, 385641. http://doi.org/10.1155/2013/385641

Tumbar, T., Guasch, G., Greco, V., Blanpain, C., Lowry, W. E., Rendl, M., & Fuchs, E. (2004). Defining the epithelial stem cell niche in skin. *Science*, *303*(5656), 359–363. http://doi.org/10.1126/science.1092436

Turksen, K., Kupper, T., Degenstein, L., Williamst, I., & Fuchs, E. (1992). Interleukin 6: Insights to its function in skin by overexpression in transgenic mice. *Proceedings of the National Academy of Sciences*, *89*(11), 5068–5072. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC49230/pdf/pnas01085-0297.pdf

Underwood, M. A., Gilbert, W. M., & Sherman, M. P. (2005). State of the Art Amniotic Fluid: Not Just Fetal Urine Anymore. *Journal of Perinatology*, *25*(5), 341–348. http://doi.org/10.1038/sj.jp.7211290

Valli, a, Rosner, M., Fuchs, C., Siegel, N., Bishop, C. E., Dolznig, H., … Hengstschläger, M. (2010). Embryoid body formation of human amniotic fluid stem cells depends on mTOR. *Oncogene*, *29*(7), 966–77. http://doi.org/10.1038/onc.2009.405

Van Bergen, T., Van de Velde, S., Vandewalle, E., Moons, L., & Stalmans, I. (2014). Improving patient outcomes following glaucoma surgery: State of the art and future perspectives. *Clinical Ophthalmology*, *8*, 857–867. http://doi.org/10.2147/OPTH.S48745

Verrecchia, F., & Mauviel, A. (2007). Transforming growth factor-β and fibrosis. *World Journal of Gastroenterology*, *13*(22), 3056–3062. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4172611/pdf/WJG-13-3056.pdf

Vowden, P., Vowden, K., Surgeon, H. C., & Trust, F. (2016). The economic impact of hard-to- heal wounds: promoting practice change to address passivity in wound management, *7*(2), 11–15.

Wallace, H. J., & Stacey, M. C. (1998). Levels of Tumor Necrosis Factor-α (TNF-α)

and Soluble TNF Receptors in Chronic Venous Leg Ulcers – Correlations to Healing Status. *Journal of Investigative Dermatology*, *110*(3), 292–296. http://doi.org/10.1046/j.1523-1747.1998.00113.x

Walter, M. N. M., Wright, K. T., Fuller, H. R., MacNeil, S., & Johnson, W. E. B. (2010). Mesenchymal stem cell-conditioned medium accelerates skin wound healing: an in vitro study of fibroblast and keratinocyte scratch assays. *Experimental Cell Research*, *316*(7), 1271–81. http://doi.org/10.1016/j.yexcr.2010.02.026

Wang, S.-H., Tsai, M.-S., Chiang, M.-F., & Li, H. (2003). A novel NK-type homeobox gene, ENK (early embryo specific NK), preferentially expressed in embryonic stem cells. *Gene Expression Patterns*, *3*, 99–103. http://doi.org/10.1016/S1567-133X(03)00005-X

Weissman, I. L. (2000a). Stem cells: units of development, units of regeneration, and units in evolution. *Cell*, *100*(1), 157–168. http://doi.org/http://dx.doi.org/10.1016/S0092-8674(00)81692-X

Weissman, I. L. (2000b). Translating stem and progenitor cell biology to the clinic: barriers and opportunities. *Science*, *287*, 1442–1446. http://doi.org/10.1126/science.287.5457.1442

Wetzler, C., Kempfer, H., Stallmeyer, B., Pfeilschifter, J., & Frank, S. (2000). Large and Sustained Induction of Chemokines during Impaired Wound Healing in the Genetically Diabetic Mouse: Prolonged Persistence of Neutrophils and Macrophages during the Late Phase of Repair. *Journal of Investigative Dermatology*, *115*(2), 245–253. http://doi.org/10.1046/j.1523-1747.2000.00029.x

WHO. (2016). Global Report on Adult Learning Executive Summary. Retrieved from http://apps.who.int/iris/bitstream/10665/204874/1/WHO_NMH_NVI_16.3_eng.pdf?ua=1

Williams RL, Hilton DJ, Pease S, Willson TA, Stewart CL, Gearing DP, Wagner EF, Metcalf D, Nicola NA, G. N. (1988). Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. *Nature*, *336*, 684–687. Retrieved from https://www.nature.com/nature/journal/v336/n6200/pdf/336684a0.pdf

Witkowska-Zimny, M. (2011). Dental Tissue as a Source of Stem Cells: Perspectives for Teeth Regeneration. *Journal of Bioengineering and Biomedical Sciences*, *1*(S2), 2–5. http://doi.org/10.4172/2155-9538.S2-006

Wu, Y., Chen, L., Scott, P. G., & Tredget, E. E. (2007a). Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells*, *25*(10), 2648–2659. http://doi.org/10.1634/stemcells.2007-0226

Wu, Y., Wang, J., Scott, P. G., & Tredget, E. E. (2007b). Bone marrow-derived stem

cells in wound healing: A review. *Wound Repair and Regeneration*, *15*, 18–26. http://doi.org/10.1111/j.1524-475X.2007.00221.x

Yadav, P. S., Mann, A., Singh, V., Yashveer, S., Sharma, R. K., & Singh, I. (2011). Expression of Pluripotency Genes in Buffalo (Bubalus bubalis) Amniotic Fluid Cells. *Reproduction in Domestic Animals*, *46*(4), 705–711. http://doi.org/10.1111/j.1439-0531.2010.01733.x

Yang, D., Wang, W. E., Li, L., Peng, Y., Chen, P., Huang, H., … Zeng, C. (2013). The Relative Contribution of Paracine Effect versus Direct Differentiation on Adipose-Derived Stem Cell Transplantation Mediated Cardiac Repair. *PLoS ONE*, *8*(3), e59020. http://doi.org/10.1371/journal.pone.0059020

Yang, J. D., Choi, D. S., Cho, Y. K., Kim, T. K., Lee, J. W., Choi, K. Y., … Byun, J. S. (2013). Effect of amniotic fluid stem cells and amniotic fluid cells on the wound healing process in a white rat model. *Archives of Plastic Surgery*, *40*(5), 496–504. http://doi.org/10.5999/aps.2013.40.5.496

Ying, Q.-L., Nichols, J., Chambers, I., & Smith, A. (2003). BMP Induction of Id Proteins Suppresses Differentiation and Sustains Embryonic Stem Cell Self-Renewal in Collaboration with STAT3. *Cell*, *115*(3), 281–292. http://doi.org/10.1016/S0092-8674(03)00847-X

Yoon, B. S., Moon, J.-H., Jun, E. K., Kim, J., Maeng, I., Kim, J. S., … You, S. (2010). Secretory profiles and wound healing effects of human amniotic fluid-derived mesenchymal stem cells. *Stem Cells and Development*, *19*(6), 887–902. http://doi.org/10.1089/scd.2009.0138

You, Q., Cai, L., Zheng, J., Tong, X., Zhang, D., & Zhang, Y. (2008). Isolation of human mesenchymal stem cells from third-trimester amniotic fluid. *International Journal of Gynecology & Obstetrics*, *103*(2), 149–152. http://doi.org/10.1016/j.ijgo.2008.06.012

You, Q., Tong, X., Guan, Y., Zhang, D., Huang, M., Zhang, Y., & Zheng, J. (2009). The biological characteristics of human third trimester amniotic fluid stem cells. *Journal of International Medical Research*, *37*(1), 105–112. http://doi.org/10.1177/147323000903700112

Zhang, J., Wang, X., Chen, B., Xiao, Z., Li, W., Lu, Y., & Dai, J. (2010). The human pluripotency gene NANOG/NANOGP8 is expressed in gastric cancer and associated with tumor development. *Oncology Letters*, *1*, 457–463. http://doi.org/10.3892/ol

Zhao, J., Hu, L., Liu, J., Gong, N., & Chen, L. (2013). The Effects of Cytokines in Adipose Stem Cell-Conditioned Medium on theMigration and Proliferation of Skin Fibroblasts In Vitro. *BioMed Research International*, 1–11.

Zhou, B. R., Xu, Y., Xu, Y., Guo, S. L., Wang, Y., Zhu, F., … Luo, D. (2013). The effect of conditioned media of Adipose-derived stem cells on wound healing after ablative fractional carbon dioxide laser resurfacing. *BioMed Research*

International, *2013*, 1–9. http://doi.org/10.1155/2013/519126

Zhou, R., Tardivel, A., Thorens, B., Choi, I., & Tschopp, J. (2010). Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nature Immunology*, *11*(2), 136–140. http://doi.org/10.1038/ni.1831