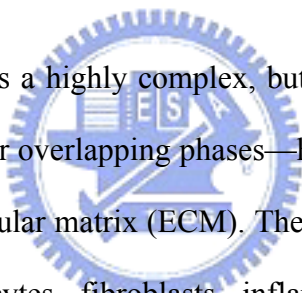


Introduction

Placental extract has been applied on many diseases, such as rheumatoid arthritis (Rosenthal M, 1982), acute first or second degree radiodermatitis (Bigliardi P, 1982), alopecia (Hauser GA, 1982), psoriasis (Bertone C et al., 1982) and wound healing (Orecchia G et al., 1984). We are interested in making use of the disposable materials, placenta, on wound healing. However, it had been demonstrated that porcine placental extracts would accelerate wound healing on rats with thermal injury (Wu CH et al., 2003). We have attempted to understand porcine and human placental extracts' function in vitro fully, and find the best extraction method for wound healing.

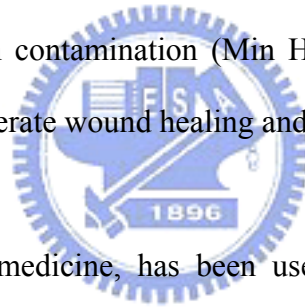


Cutaneous wound healing is a highly complex, but orchestrated cascade of events which can roughly be divided into four overlapping phases—hemostasis, inflammation, proliferation and remodeling of the extracellular matrix (ECM). These events are governed by a number of different cell types (keratinocytes, fibroblasts, inflammatory and endothelial cells), and involve several cellular phenomena such as migration, proliferation, adhesion, phenotypic differentiation, etc. (Raghow R et al., 1994; Gailit J and Clark RA, 1994). Each phase of wound healing is distinct, although the wound healing process is continuous, with each phase overlapping the next. It is characterized by proliferation and migration of various cell types as well as expression of ECM components. All these processes are controlled by a variety of soluble mediators, including cytokines and growth factors (Martin P, 1997). As the blood components spill into the site of injury, the platelets come into contact with exposed collagen and other elements of the ECM. This contact triggers the platelets to release clotting factors as well as essential growth factors and cytokines such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF- β), and other peptides (Falanga V et al., 1992). The

formation of a clot then serves as a reservoir of growth factors and cytokines. Following hemostasis, the neutrophils then enter the wound site and begin the critical task of phagocytosis to remove foreign materials, bacteria and damaged tissue. As part of this inflammatory phase, the macrophages appear and continue the process of phagocytosis as well as releasing more PDGF and TGF- β . Once the wound site is cleaned out, fibroblasts migrate into begin the proliferation phase and deposit new ECM, form granulation tissue. Fibroblasts and keratinocyte migrate over the wound and subsequently proliferation, which finally leads to complete wound re-epithelialization (Clark RAF, 1991). During the final remodeling phase, the new collagen matrix then becomes cross-linked and organized (Diegelmann RF and Evans MC, 2004).

Moreover, the formation of new blood vasculature from preexisting vessels, a process called angiogenesis, is also essential to wound healing (Folkman J and Shing T, 1992; Carmeliet P, 2003). Newly formed blood vessels participate in provisional granulation tissue formation and provide nutrition and oxygen to growing tissues. In addition, inflammatory cells require the interaction with and transmigration through the blood vessel endothelial basement membrane to enter the site of injury. In normal adult tissues, with the exception of hair cycle and female reproductive cycle of the uterus and the ovaries, the blood vasculatures remain quiescent but have the capacity to initiate angiogenesis during wound repair. Angiogenesis, in response to tissue injury, is a dynamic process that is highly regulated by signals from both serum and the surrounding specialized ECM environment (Risau W et al., 1997). In response to the injury, microvascular endothelial cells initiate an angiogenic process consisting of induction of microvascular hyperpermeability, local degradation of their basement membrane, migration and formation of granulation tissue, reconstruction of basement membrane, formation of new blood vessel, stabilization, and eventually regression and involution of the newly formed vasculature as tissue remodeling (Marx M et al., 1994).

Wound healing process commonly leads to scarring if the dermis is wounded (Stocum DL et al., 1995). Scarring is one of the major problems confronting surgical recovery and wound healing (Stocum DL et al., 1995). Scar formation and fibrosis often cause reduced functional restoration after surgery or trauma (Cass DL et al., 1997). Abnormal scarring can cause pain, cosmetic deformities, and wound contracture, all of which can prolong a patient's hospital stay and limit his ability to function even long after discharge (Neely AN et al., 1999). Scar tissue at its maximum tensile strength is only 70% as strong as normal skin (Clark and Singer, 2000). It has been reported that the most important indicator of whether a burn wound would develop hypertrophic scars was the time required for wound to heal. Delayed wound healing results frequently development of hypertrophic scars in the burn site (Deitch EA et al., 1983). However, minimization of scar formation currently just depends on improving the healing rate by protecting the wound from contamination (Min Hu et al., 2003). We wanted to explore whether placental extract accelerate wound healing and then diminish scar formation.



Placenta, a Chinese folk medicine, has been used in clinical application for patients suffering from rheumatoid arthritis, acute first or second degree radiodermatitis, alopecia, psoriasis some surgical affections, and so on, following the studies by Filatov since 1948. During the implantation process, the embryo produces membranes that gradually attach to the endometrial epithelium and thereby establish a close relationship between fetal and maternal circulatory systems for physiologic exchange. As a result, a combined organ, the placenta, is formed. Formation of placenta includes extensive angiogenesis, which increases uterine (maternal placenta) and umbilical (fetal placenta) blood flow, and is critical for successful development of viable, healthy offspring (Reynolds LP et al., 2001). The placenta operates as a vital interface between the mother and fetus. In addition to facilitating fetal nourishment, it acts as a barrier both to potentially deleterious agents and to contact between their two immune systems. As a consequence, damage to the placenta, even on a relatively small scale,

could be very dangerous to the fetus. Therefore, wound repair mechanisms are likely to be of great importance in ensuring that an intact placental barrier is re-established as soon as possible (Watson AL et al., 1996). Moreover, these relative growth factors produced by placental tissues themselves.

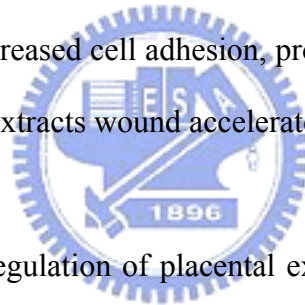
Due to wound healing process is controlled by a variety of cytokines and growth factors, such as TGF- β 1, keratinocyte growth factor (KGF), fibroblast growth factors (FGFs) and stimulators of cell growth are found to be present in placenta, placenta is an extremely rich reservoir of bioactive molecules. Vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and FGF are major growth factors of the placenta (Morrish et al., 1987; Hamai et al., 1998; Amemiya et al., 1994; Reynolds and Redmer, 2001). We are interested in placental extracts applied on wound healing. In accordance with wound healing process, growth factors have chemotactic activities that attract inflammatory cells and fibroblasts into the wound, act as mitogens to stimulate cellular migration, proliferation, and angiogenesis, and have a profound effect on ECM. Wound healing is a complex biological process that requires cellular interactions between a variety of cells, including fibroblasts, myofibroblasts, smooth muscle cells, endothelial cells, keratinocytes and immune cells. These interactions are mediated by numerous factors such as growth factors, hormones, blood components and second messengers. The significant growth factors in relation to wound healing were indicated in Table 1. The clinical use of growth factors to stimulate the healing of wounds is currently being investigated. Several growth factors, including PDGF, FGF-2, insulin growth factor (IGF) and KGF, have been used in clinical trials, and PDGF is currently approved for use in human medicine (Grazul-Bilska AT et al., 2003). We determined some growth factors involved in the wound healing process in our placental extracts. For example, the well-established maker TGF- β 1 is thought to play most pivotal and coordinating role (Postlethwaite AE et al., 1987; Wahl SM et al., 1987) in wound healing. VEGF and placenta

growth factor (PIGF) are potent angiogenesis growth factors. VEGF is the major angiogenic factor that regulates degradation of the ECM, and endothelial cell migration and proliferation, as well as angiogenesis (Detmar M et al., 1996). PIGF also stimulates endothelial cell migration and proliferation (Odorisio T, 2002). KGF, which stimulates wound re-epithelialization, is a potent mitogen for keratinocytes (Nanney LB, 1990; Marchese C et al., 1995). Furthermore, chronic ulcers are known to have reduced levels of PDGF, bFGF, EGF, and TGF β compared with acute wounds (Higley HR et al., 1995). Our tactic was providing various growth factors or other unknown substances in placental extract on wound healing. Accordingly, we believe that placental extract is potential to be applied on accelerating wound healing.

In Taiwan, swine industry is prosperous. It's easy to get and quality control the porcine placenta. Moreover, the gestation of lying-in sow with two placentas is shorter than human. We chose porcine placenta for major wound healing study. We also estimated human placental extract for wound healing. Considering hormones dissolved in organic solvent have been studied thoroughly, we focused on placental extracts dissolved in aqueous solution.

Because fibroblasts and keratinocytes are the two major cell types in the dermis and epidermis responsible for wound healing, they are selected to determine whether placental extracts affect them in wound healing. Fibroblasts play a central role in wound healing by producing and maintaining the connective tissue matrix and eventually contracting the newly formed connective tissue to bring together the edges of the wound (Muratorea O et al., 1997). Keratinocytes are the dominant epithelial cell type in the epidermis, a complex squamous epithelium that form the outer surface of the skin (Priestley GC, 1993) and that is separated from the underlying dermis by the basement membrane. Angiogenesis, formation of new blood vessels from existing endothelium, also occurs during wound repair (Folkman J and

Klagsbrun M, 1987; Folkman J, 1991, 1992; Breier G et al., 1997; Risau W, 1997). The process of angiogenesis consists of basement membrane degradation, migration and proliferation of endothelial cell. A number of cytokines and growth factors are known to modulate angiogenesis. Among these factors, bFGF and VEGF appear to be potent angiogenesis inducers (Seghezzi G et al., 1998). Because fibroblasts, keratinocytes, and endothelial cells migration and proliferation are important parameters in wound healing, we evaluated the activity of placental extracts by cell proliferation and migration assay. For most tissue engineering applications, it is a general requirement that cells adhere to the particular support material or scaffold that is being used. Cell attachment has been shown to strongly influence cell proliferation, migration, differentiation, and ECM production (Griffith L, 2000). We also explored whether placental extracts influence cell adhesion. Our data showed that placental extracts treatment increased cell adhesion, proliferation and migration. These results suggest that porcine placental extracts would accelerate wound healing effectively.



Further, we explored the regulation of placental extracts on ECM because mesenchymal cell migration and tissue remodeling during wound healing require the controlled degradation of ECM. These processes are partly regulated by extracellular proteases, matrix metalloproteinase (MMPs) (Soo et al., 2000). MMPs are a family of enzymes that degrade and remodel the ECM and, play a central role in the wound healing process (Ravanti L et al., 2000). In general, MMPs are not constitutively expressed in skin but are induced temporarily in response to exogenous signals such as various cytokines, growth factors, cell matrix interactions and altered cell-cell contacts (Mauviel A et al., 1993). MMPs are secreted as inactive zymogens and can degrade various components of scar tissue ECM. Therefore, the accumulation and organization of matrix components, and their modeling by MMPs, are instrumental for wound healing and associated scar formation. The MMPs are produced by a number of cell types involved in wound repair, including fibroblasts, macrophages,

endothelial cells, and keratinocytes (Nagase H et al., 1997). MMP-2 (72 kDa type IV collagenase, gelatinase A) and MMP-9 (92 kDa type IV collagenase, gelatinase B) have been implicated in various aspects of tissue maintenance and wound healing (Ravanti L, and Kahari VM, 2000). MMP-2 is able to release fibronectin fragments that stimulate cell migration (Fukai F et al., 1995). MMP-9 appears to be particularly important for keratinocyte migration during re-epithelialization (Salo et al., 1994). However, MMP-9 activity is low or undetectable in hypertrophic scar where collagen is excessive (Neely AN et al., 1999). Matrix digestion by MMPs, including MMP-1, MMP-2, and MMP-9, is a pre-requisite for endothelial cell activation and angiogenesis. Harmonized regulation of ECM production and degradation is critical in the process of normal wound healing. Excess healing leads to excess scar and healing itself requires some MMP activity to break down damaged tissue, making room for migration of new tissue formation. We tried to ascertain whether the alterations of MMP-1, MMP-2, and MMP-9 are associated with wound healing influenced by placental extracts.

