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# Electrospun Biomaterial Scaffolds for Skin Regenerative Medicine

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**Abstract:** Skin plays a crucial role in protecting the human body against the infections, environment and dehydration. Skin contains two main layers: a keratinized, stratified epidermis and an underlying thick layer of collagen-rich dermal connective tissue. Loss of large portions of skin integrity from wounds, burns or disease may result in significant disabilities or even death. So, treatment of skin injuries by wound management, skin transplantation and stem cell therapy can largely protect the patient from various bad outcomes. However, treatment of skin injuries has been a challenging task for surgeons because of multiple limitations. Allogenic and autologous keratinocyte grafts, accellular biological matrices and cellular matrices including biological substances are different forms of tissue-engineering skin injuries treatments.

**Key words:** Scaffolds, dehydration, keratinized, skin transplantation, stem cell therapy

## INTRODUCTION

The World Health Organization (WHO) estimates that over 300,000 deaths are attributable to fire-related burn injuries with millions more suffering from the partly devastating physical and emotional consequences thereof, annually (Burke *et al*., 1981). A further 6.5 million individuals suffer from chronic skin ulcers caused by prolonged pressure, venous stasis or diabetes mellitus (Jones *et al*., 2002). The holistic goals of modern cutaneous wound care consist of rapid wound excision and closure with a functionally intact and aesthetically pleasing outcome. Currently, available treatment options are lacking in establishing both functional and cosmetic satisfaction. Wound contracture and scarring, properties that can misleadingly be thought accountable for faster wound healing should be avoided in terms of wound regeneration. Skin wounds are a major social and financial burden. However, current treatments are suboptimal. The gradual comprehension of the finely orchestrated nature of intercellular communication has stimulated scientists to investigate Growth Factor (GF) or Stem Cell (SC) incorporation into suitable scaffolds for local delivery into wound beds in an attempt to accelerate healing. Min and colleagues carried out a series of studies to investigate the potential of silk electrospun matrices for accelerating early stages wound healing (Min *et al*., 2004, 2006). The spreading and adhesion of normal human fibroblasts and keratinocytes on methanol-treated silk fibroin nanofibers alone or precoated with ECM proteins (collagen type I, fibronectin and laminin) was evaluated. For keratinocytes but not fibroblasts by coating with collagen I, cell spreading and cell adhesion were promoted. Laminin coating stimulated cell spreading but not cell attachment. Cell adhesion and spreading on fibronectin-coated silk matrices was different from that on BSA-coated matrices (Min *et al*., 2004). In wound healing studies with electrospun microfibers, silk nanofibers and films, nanofibrous matrices promoted human oral keratinocyte adhesion and spreading, especially when collagens type I coating was included (Min *et al*., 2004). Fibroblast spreading was also significantly improved when silk matrices were treated with water vapor rather than methanol (Min *et al*., 2006). Thus, electrospun silk nanofibrous matrices treated with water vapor followed by collagen I coating was the best candidate for a skin tissue template or wound dressing.

In another study, due to the wound healing effects of chitin it was blended with silk fibroin to fabricate composite fibrous scaffolds for skin tissue engineering (Okamoto *et al*., 2002). The chitin/silk fibroin blends at varied ratios were electrospun into nanofibrous matrices and evaluated for initial cell attachment and spreading (Park *et al*., 2006). Adhesion of keratinocytes was increased on chitin/silk blend matrices compared to pure chitin matrices although, the same result was achieved for fibroblasts. Yoo *et al*. (2008) also, fabricated chitin/silk hybrid matrices at various blend ratios using side by side

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electrospinning and compared these to chitin/silk blend matrices for keratinocyte adhesion and spreading. In Another study, Yoo *et al.* (2008) investigated the potential of collagen/silk blend electrospun matrices for skin tissue engineering. Surprisingly, pure collagen and pure silk fibroin matrices exhibited better results in terms of human keratinocyte attachment and spreading than collagen/silk blend or hybrid matrices irrespective of the mixing ratios. On the other hand all matrices had similar effects on human fibroblast attachment and spreading. When collagen/silk hybrid matrices and collagen/silk blend matrices with equal component ratios were compared, hybrid matrices consistently supported better cell attachment and spreading (Wang *et al*., 2006).

In this study, researchers stress the importance of a bottom-up approach in scaffold fabrication and electrospinning to enable controlled factor incorporation as well as production of complex scaffold micro and nanostructures resembling that of natural extracellular matrix. Also, this review provides a critical evaluation of the status quo of current research into GF and SC therapy and subsequent future prospects including benefits and possible long-term dangers associated with their use.

## TISSUE ENGINEERING AND NANO SCAFFOLDS FOR TREATMENT OF THE SKIN LESIONS

A 3D supporting framework should provide a template for tissue regeneration while simultaneously preventing wound bed contraction throughout the first stages of healing (Chevallay and Herbage, 2000). The framework or scaffold should further serve as a platform for cellular adhesion, localization and differentiation (Kim and Mooney, 1998). A focus on the multitude of different scaffold materials and fabrication techniques but has been extensively reviewed elsewhere and is beyond the scope of this study (Edalat *et al*., 2012).

Scaffold materials can be of natural, composite or synthetic origin and engineered using a multitude of approaches including electrospinning, molecular self-assembly, porogen leaching and phase separation. The mixing of materials from different classes to obtain best composite scaffolds seems particularly promising because individual limitations of single material scaffolds (e.g., poor mechanical properties) are often overcome by the composite nature (Kannan *et al*., 2007). The ideal skin regeneration scaffold should actively direct tissue formation and prevent scarring. Thus, much focus has been channeled into creating suitable biomimetic surface micro and nanostructures that can act as delivery vehicles for Growth Factor (GFs) or SCs. The synergistic tissue regenerating effects of a smart scaffold cocktail comprising, favourable scaffold surface patterns, GFs and SCs have the realistic potential of overcoming current barriers and enabling fast and complete skin regeneration.

During natural wound healing, dynamic and reciprocal interactions between components of the Extra Cellular Membrane (ECM) and surrounding cell-signaling molecules are responsible for the expression of Growth Factor (GFs) and cytokines. These interactions elicit cellular responses that ultimately lead to new tissue generation. Overwhelming activation of the inflammatory system and prolific recruitment of contractile cells is thought to stem from prehistoric adaptations of human skin to close irregular and often contaminated wounds as rapidly as possible to prevent microorganism invasion and potentially lethal infections (Thorne, 2007). Unfortunately, this response typically leads to scar formation often resulting in disfigurement and functional disability.

Regenerative medicine aims at recuperating lost tissues by guiding cell growth and restoring original tissue architecture. This requires the presence of a scaffold because isolated cells are unable to re-establish their native structures due to a lack in extracellular guidance. Bioactivation is one of efforts for increasing their effectiveness and a simple structural framework to a delivery vehicle for Gfs, cytokines orgenes (Yang *et al*., 2011; Barrientos *et al*., 2008). So, tissue regeneration can be actively promoted. This should however, not discourage research into more suitable scaffold architectures because accumulating evidence highlights the importance of biocompatible scaffold materials, appropriate pore sizes and cell growth promoting surface topographies (Wong *et al*., 2010; Yildirimer *et al*., 2011). On the other hand, cells cultured on nano fibrous structures, demonstrated a phenotype resembling that of cells growing within natural environments perhaps because of the close architectural approximation of nano fibrous materials to natural ECM and collagen fibrils which themselves exhibit nanometer dimensions. Thus, a natural cell environment can be feigned and cells guided along normal morphogenic lines.

The burden of cutaneous wounds is immense in both personal and financial terms. Clinically effective and feasible treatment strategies are still lacking despite various skin substitutes being under thorough investigation (Yildirimer *et al*., 2012).

The cooperation of nanotechnology combined with the SC technology and the ever-increasing appreciation of cell-signaling pathways in both fetal and adult wound healing models have opened up new avenues to accelerated healing for precise biotechnological wound

bed manipulations. Approved treatment strategies for skin wounds mostly aim to replace lost tissues rather than support intrinsic self-healing mechanisms. Technological advances has helped scientists to manipulate precisely scaffold materials and engineering strategies within nanometer dimensions to create nano topographies that mimic the natural ECM.

The incorporation of SCs, GFs and nanoparticles into scaffolds promotes the intricate interplay between naturally occurring cell-signaling factors to achieve full tissue regeneration. With such fast and current developments in nanotechnology and biomedical sciences, researchers are continuously improving skin regeneration and repair. The future trend of regenerative medicine in general and tissue-engineering of skin in particular lies in: the comprehension of intricate intercellular biochemical communications; the engineering of scaffold structures on a micro and nanodimension and the integration of GFs and SCs into such scaffolds to obtain a bioactive cocktail capable of active guidance in skin regeneration. Despite the presence of realistic benefits and dangers associated with GF or SC supplementation, ongoing research into their exploitation is fundamental if regenerative medicine is to have a future.

In choosing the material to be used for a scaffold, a wide range of options exists-natural and synthetic materials and composites of two or more from the same class or different classes of materials; the advantages and disadvantages of using that material must be known in addition to its suitability for the desired application.

Naturally-derived materials are often purified Extracellular Matrix (ECM) proteins (collagen, gelatin (Chen *et al*., 2012), laminin, hyaluronic acid) or a mixture (Matrigel1). Other sources may be from plant and animal constituents (silk, agarose and chitosan). Alternatively, decellularized organs that retain the ECM and architecture of tissues have been used to engineer blood vessel (Quint *et al*., 2011), heart (Ott *et al*., 2008), lung (Petersen *et al*., 2010), liver (Uygun *et al*., 2010) and bone (Grayson *et al*., 2010). The advantages of natural materials are their biological activity and biocompatibility. Synthetic materials, on the contrary, overcome the disadvantages posed by their natural counterparts-mainly their manufacturing and processing variability and inability to control their physico-chemical properties. Additionally, synthetic materials provide a blank slate with absence of biological activity that may be modified through biochemical means (discussed in Biochemical modulation of materials section) (Zhu, 2010). Overall, researchers support the argument that a bottom-up approach: enables tight control over important micro and nanostructures within scaffold architecture and facilitates incorporation of appropriate concentrations of bioactive factors as well as SCs.

More often than expected, single-component materials do not meet the requirements needed for a cellular scaffold. For instance, they may lack the desired mechanical properties, electrical activity or cell-matrix interactions. Composite materials may be used to overcome these limitations. While mixing materials from the same class (Shin *et al*., 2011) will provide a degree of modulation combining materials from different classes will generate a greater measure of control over its properties. For instance bone is composed of collagen (a polymer) and hydroxyapatite Nano crystals (a ceramic) hence, polymer/ceramic composites have been widely used in bone tissue engineering (Zhang *et al*., 2009). Hydrogels are another case in point they are a cross-linked network of monomers, oligomers or polymers that contain 90-95% water in volume and structurally resemble the ECM (Tibbitt and Anseth, 2009). However, they often lack the mechanical strength needed for certain tissue engineering applications. In a research by Shin *et al*. (2012) gelatin methacrylate hydrogels which favor cell attachment and spreading but lack strong mechanical properties were reinforced with carbon nanotubes which resulted in a composite with increased compressive modulus while material pore size and cell adhesiveness remained the same. Furthermore, carbon nanotube based composites have been used to direct differentiation of mesenchymal stem cells toward the osteogenic lineage (Namgung *et al*., 2011) increase connexin 43 expression of cardiac constructs (You *et al*., 2011) and enhance the electrical activity of neural tissues (Cellot *et al*., 2008) given the electrical conduction properties of carbon nanotubes.

In designing a scaffold, it is ideal that the scaffold, over an intended period of time should degrade and be replaced with naturally deposited ECM and the newly formed tissue. In this regard, linear aliphatic polyesters such as poly (lactic acid) and poly (glycolic acid) have been routinely used owing to their biodegradability given the susceptibility of their ester bonds to hydrolysis and ability to fine-tune their degradation rate. Alternatively, non-biodegradable materials such as Poly Ethylene Glycol (PEG) can be incorporated with Matrix Metalloproteinase (MMP) sensitive peptides to make them physiologically degradable. The addition of such peptides has been shown to directly affect gene expression as shown by the increased maturation of cardio progenitors via increase in myosin heavy chain-positive cells when grown on MMP-sensitive gels (Kraehenbuehl *et al*., 2008). Another important consideration in cellular scaffold fabrication is scaffold structure. In the past, emphasis was placed on macro porous structures to facilitate mass transfer of vital molecules. These scaffolds were often fabricated with microspheres, salt leaches or gas foams (Annabi *et al*.,

2009). However, the micron-scale dimensions of these material structures do not recapitulate the nanometer-scale fibrillar aspect of the structure of ECM. To generate these nano fibers, techniques such as electrospinning (Sill and Recum, 2008) molecular self-assembly (Hartgerink *et al*., 2001; Cui *et al*., 2010) and phase separation (Liu and Ma, 2009) have been employed. Hydrogels, discussed previously are a class of materials that have proven to be particularly biomimetic and are now widely used in the biological and medical fields (Slaughter *et al*., 2009).

## SKIN, NANO SCAFFOLDS AND GROWTH FACTORS

**Epidermal Growth Factor (EGF):** EGF is implicated in keratinocyte migration, fibroblast proliferation and differentiation and granulation tissue formation. EGF significantly enhances wound healing (Alemdaroglu *et al*., 2008) as well as the tensile strength of the resultant ECM (Throm *et al*., 2010). Prolonged exposure to EGF yielded a 200% increase in tensile strength when liposome-encapsulated EGF was delivered into murine dorsal cutaneous wounds (Brown *et al*., 1988). Similarly, EGF contained within a gelatine sheet applied to dorsa of hairless dogs accelerated wound closure and healing of superficial and partial-thickness wounds (Tanaka *et al*., 2005). Current challenges regarding the delivery of EGF at physiologically relevant concentrations and durations still prevail due to its rapid breakdown within the wound environment, encouraging research into effective immobilization and delivery techniques. For example, biodegradable microspheres that contain EGF provide sustained EGF delivery and hence more effective wound healing in a rabbit dorsal skin wound model (Ulubayram *et al*., 2001). Despite such encouraging results, supplemental EGF has a mitogenic effect upon cells and has been implicated in the spread of epithelial malignancies (Hardwicke *et al*., 2008). However, the beneficial effects of EGF in the wound healing process should not be denied thus requiring further research.

**Basic Fibroblast Growth Factor (BFGF):** FGF comprises a large family of mitogens that are actively involved in the processes of wound healing, embryonic development, angiogenesis and tumor progression (Thisse and Thisse, 2005; Dailey *et al*., 2005; Beenken and Mohammadi, 2009). Both acidic Fibroblast Growth Factor (aFGF) and bFGF are found within the wound fluid at the earliest stages of healing (Nissen *et al*., 2003). aFGF like bFGF is a potent mitogen and chemo-attractant for vascular endothelial cells, dermal fibroblasts and epidermal keratinocytes. In the initial phases of wound healing, bFGF participates by activating local macrophages and can still be identified within the healing tissue during the remodeling phase that occurs several weeks after injury (Nissen *et al*., 2003). bFGF accelerates neovascularization as shown in a rabbit ear wound healing model in which wounds supplemented with exogenous bFGF healed significantly faster compared to untreated controls (Komori *et al*., 2005).

**Vascular Endothelial Growth Factor (VEGF):** The VEGF family encompasses VEGF-A, VEGF-B, VEGFC, VEGF-D and PlGF. During wound healing, VEGF-A is highly expressed by keratinocytes within the wound bed to promote new blood vessel formation essential for tissue regeneration (Drinkwater *et al*., 2002) and re-epithelialization of wounds (Wise *et al*., 2012; Brem *et al*., 2009). VEGF is the major angiogenesis-promoting GF due to its combined ability to stimulate endothelial cell proliferation and migration (Ferrara and Henzel, 1989) basement membrane degradation (Unemori *et al*., 2005), tubular and luminal structure formation (Koolwijk *et al*., 1996) increased vascular permeability (Bates and Curry, 1996) and new vessel formation (Connolly *et al*., 1989). In a diabetic mouse model, minicircle plasmid DNA encoding VEGF was successfully transfected into proliferating cells within wounded tissue, resulting in a high level of VEGF expression (Kwon *et al*., 2012). Wound healing rates were significantly accelerated in plasmid-exposed injuries compared to those exposed to empty vehicles. Similarly, vector-mediated VEGF transfer onto experimental murine burn wounds increased angiogenesis as well as epithelial proliferation and ECM maturation (Galeano *et al*., 2003).

**Keratinocyte Growth Factor (KGF):** KGF is expressed within the dermis and hypodermis below the wound whereas the KGF receptor is predominantly found on epithelial cells of the epidermis, suggesting a paracrine mediation of epithelial cell growth. Several animal and clinical studies have demonstrated the cytoprotective and epithelial regenerative properties of KGF (Wu *et al*., 2011; Yazbeck *et al*., 2011; Spielberger *et al*., 2004). Such favourable effects depend on several mechanisms including cell proliferation, migration, differentiation, survival, DNA repair and detoxifying enzyme induction which collectively act to strengthen the integrity of the epithelium (Finch and Rubin, 2004). During the initial 24 h of normal human wound healing, KGF expression is up regulated 100 fold and remains elevated for several days (Marchese *et al*., 1995). This up regulation in KGF is significantly dampened in genetically diabetic and glucocorticoid-treated mice (Brauchle *et al*., 1995; Werner *et al*., 1994).

**Insulin-like Growth Factor-1 (IGF-1):** High levels of IGF-1 found within cutaneous wounds have been shown to accelerate epidermal wound healing and inhibit apoptosis pathways (Dasu *et al*., 2003; Spies *et al*., 2001) whereas reduced expression of IGF-1 is associated with retarded wound healing (Gartner *et al*., 1992). For example, IGF-1 receptor knockout mice (IGF-1r$^{-/-}$) exhibited skin hypotrophy with fewer and smaller hair follicles (Liu *et al*., 1993). Wound healing by re-epithelialization was accelerated by local overexpression of IGF-1 whereas no effects were observed on the underlying dermis (Semenova *et al*., 2008). *In vitro* studies however have found evidence for the stimulatory effects of IGF-1 on collagen and ECM production (Daian *et al*., 2003).

**Platelet-Derived Growth Factor (PDGF):** PDGF is involved throughout all stages of normal wound healing. PDGF is released by degranulation platelets and activated macrophages within the wound fluid. Later in the proliferative stage, PDGF is responsible for the differentiation of fibroblasts into their contractile phenotype, the myofibroblasts which through attachments of their filopodia to components of the ECM, drag or contract the tissues together (Clark, 1993). Several animal studies have demonstrated accelerated wound closure in normal and pathophysiological states when the wound bed was supplemented with exogenous PDGF (Uhl *et al*., 2003).

**Transforming Growth Factor-b (TGF-b):** TGF-bexists in three isoforms TGF-b1, TGF-b2 and TGF-b3 which are all involved in the process of wound healing. After acute injuries, TGF-b1 is highly expressed by keratinocytes, platelets, monocytes, fibroblasts and macrophages (Uhl *et al*., 2003). TGF-b acts in both autocrine and paracrine manners, inducing its own synthesis by target cells and activating nearby cells to synthesize and release other Gfs involved in the healing process (Murphy *et al*., 2011). The autocrine action of TGF-b1 by fibroblasts sustains their activity beyond the initial inflammatory stimulus (Schmid *et al*., 1998) and is postulated to play a key role in myofibroblast differentiation (Tomasek *et al*., 2003). TGF-b2 expression is related to wound contracture and excessive collagen deposition but has also been demonstrated to be a causative factor in scar formation (Shah *et al*., 1995).

## CONCLUSION

The ultimate purpose of tissue-engineered skin grafts is to enable complete and natural, albeit accelerated, wound regeneration. Here, researchers review and critically appraise current research efforts concerned with dermal regeneration scaffolds incorporating bioactive elements to promote neovascularization and tissue regeneration. Researchers also, touch upon the most recent advances in the field of nano-scaled tissue engineering because cellular behavior is significantly influenced by the surface nano topography of the scaffold that promotes cellular adherence, differentiation and proliferation, mimicking natural Extra Cellular Matrix (ECM).

## REFERENCES

Alemdaroglu C., Z. Degim, N. Celebi, M. Sengezer, M. Alomeroglu and A. Nacar, 2008. Investigation of epidermal growth factor containing liposome formulation effects on burn wound healing. J. Biomed. Mater. Res. A, 85: 271-283.

Annabi, N., S.M. Mithieux, E.A. Boughton, A.J. Ruys, A.S. Weiss and F. Dehghani, 2009. Synthesis of highly porous crosslinked elastin hydrogels and their interaction with fibroblasts<i> *in vitro*. Biomaterials, 30: 4550-4557.

Barrientos, S., O. Stojadinovic, M.S. Golinko, H. Brem and M. Tomic-Canic, 2008. Growth factors and cytokines in wound healing. Wound Repair Regen., 16: 585-601.

Bates, D.O. and F.E. Curry, 1996. Vascular endothelial growth factor increases hydraulic conductivity of isolated perfused microvessels. Am. J. Physiol., 271: H2520-H1528.

Beenken, A. and M. Mohammadi, 2009. The FGF family: Biology, pathophysiology and therapy. Nat. Rev. Drug Discov., 8: 235-253.

Brauchle, M., R. Fassler and S. Werner, 1995. Suppression of keratinocyte growth factor expression by glucocorticoids *in vitro* and during wound healing. J. Invest. Dermatol., 105: 579-584.

Brem, H., A. Kodra, M. S. Golinko, H. Entero and O. Stojadinovic *et al*., 2009. Mechanism of sustained release of vascular endothelial growth factor in accelerating experimental diabetic healing. J. Invest. Dermatol., 129: 2275-2287.

Brown, G.L., L.J. Curtsinger, M. White, R.O. Mitchell and J. Pietsch *et al*., 1988. Acceleration of tensile strength of incisions treated with EGF and TGF-beta. Ann. Surg., 208: 788-794.

Burke, J.F., I.V. Yannas, W.C. Quinby Jr, C.C. Bondoc and W.K. Jung, 1981. Successful use of a physiologically acceptable artificial skin in the treatment of extensive burn injury. Anna. Surg., 194: 413-428.

Cellot, G., E. Cilia, S. Cipollone, V. Rancic and A. Sucapane *et al*., 2008. Carbon nanotubes might improve neuronal performance by favouring electrical shortcuts. Nat. nanotechnol., 4: 126-133.

Chen, Y.C., R.Z. Lin, H. Qi, Y. Yang and H. Bae *et al*., 2012. Functional human vascular network generated in photocrosslinkable gelatin methacrylate hydrogels. Adv. Funct. Mater., 22: 2027-2039.

Chevallay, B. and D. Herbage, 2000. Collagen-based biomaterials as 3D scaffold for cell cultures: Applications for tissue engineering and gene therapy. Med. Biol. Eng. Comput., 38: 211-218.

Clark, R.A., 1993. Regulation of fibroplasia in cutaneous wound repair. Am. J. Med. Sci., 306: 42-48.

Connolly, D.T., D.M. Heuvelman, R. Nelson, J.V. Olander and B.L. Eppley *et al*., 1989. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J. Clin. Investi., 84: 1470-1478.

Cui, H., M.J. Webber and S.I. Stupp, 2010. Self-assembly of peptide amphiphiles: From molecules to nanostructures to biomaterials. Peptide Sci., 94: 1-8.

Daian, T., A. Ohtsuru, T. Rogounovitch, H. Ishihara and A. Hirano *et al*., 2003. Insulin-like growth factor-I enhances transforming growth factor-β-induced extracellular matrix protein production through the P38/activating transcription factor-2 signaling pathway in keloid fibroblasts. J. Invest. Dermatol., 120: 956-962.

Dailey, L., D. Ambrosetti, A. Mansukhani and C. Basilico, 2005. Mechanisms underlying differential responses to FGF signaling. Cytokine Growth Factor Rev., 16: 233-247.

Dasu, M. R. K., D. N. Herndon, O. Nesic and J.R. Perez-Polo, 2003. IGF-I gene transfer effects on inflammatory elements present after thermal trauma. Am. J. Physiol. Regul. Integr. Comp. Physiol., 285: R741-R746.

Drinkwater, S., A. Smith, B. Sawyer and K. Burnand, 2002. Effect of venous ulcer exudates on angiogenesis *in vitro*. Br. J. Surg., 89: 709-713.

Edalat F., I. Sheu, S. Manoucheri and A. Khademhosseini, 2012. Material strategies for creating artificial cell-instructive niches. Curr. Opin. Biotechnol., 23: 820-825.

Ferrara, N. and W.J. Henzel, 1989. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem. Biophys. Res. Commun., 161: 851-858.

Finch, P.W. and J.S. Rubin, 2004. Keratinocyte growth factor/fibroblast growth factor 7, a homeostatic factor with therapeutic potential for epithelial protection and repair. Adv. Cancer Res., 91: 69-136.

Galeano, M., B. Deodato, D. Altavilla, G. Squadrito and P. Seminara *et al*., 2003. Effect of recombinant adeno-associated virus vector-mediated vascular endothelial growth factor gene transfer on wound healing after burn injury. Crit. Care Med., 31: 1017-1025.

Gartner, M.H., J.D. Benson and M.D. Caldwell, 1992. Insulin-like growth factors I and II expression in the healing wound. J. Surgical Res., 52: 389-394.

Grayson, W.L., M. Frohlich, K. Yeager, S. Bhumiratana and M.E. Chan*et al*., 2010. Engineering anatomically shaped human bone grafts. Proc. Nat. Acad. Sci., 107: 3299-3304.

Hardwicke, J., D. Schmaljohann, D. Boyce and D. Thomas, 2008. Epidermal growth factor therapy and wound healing-past, present and future perspectives. Surgeon, 6: 172-177.

Hartgerink, J. D., E. Beniash and S. I. Stupp, 2001. Self-assembly and mineralization of peptide-amphiphile nanofibers. Science, 294: 1684-1688.

Jones, I., L. Currie and R. Martin, 2002. A guide to biological skin substitutes. Br. J. Plast. Surg., 55: 185-193.

Kannan, R. Y., H. J. Salacinski, J. Ghanavi and A. Narula *et al*., 2007. Silsesquioxane nanocomposites as tissue implants. Plast. reconstructive surg., 119: 1653-1662.

Kim, B.S. and D.J. Mooney, 1998. Development of biocompatible synthetic extracellular matrices for tissue engineering. Trends Biotechnol., 16: 224-230.

Komori, M., Y. Tomizawa, K. Takada and M. Ozaki, 2005. A single local application of recombinant human basic fibroblast growth factor accelerates initial angiogenesis during wound healing in rabbit ear chamber. Anesth. Analg., 100: 830-834.

Koolwijk, P., M. van Erck, W. de Vree, M.A. Vermeer and H.A. Weich *et al*., 1996. Cooperative effect of TNFalpha, bFGF and VEGF on the formation of tubular structures of human microvascular endothelial cells in a fibrin matrix. Role of urokinase activity. J. Cell Biolo., 132: 1177-1188.

Kraehenbuehl, T. P., P. Zammaretti, A. J. V. der Vlies, R.G. Schoenmakers, M.P. Lutolf, M.E. Jaconi and J.A. Hubbell, 2008. Three-dimensional extracellular matrix-directed cardioprogenitor differentiation: Systematic modulation of a synthetic cell-responsive PEG-hydrogel. Biomaterials, 29: 2757-2766.

Kwon, M. J., S. An, S.Choi, K. Nam and H.S. Jung *et al*., 2012. Effective healing of diabetic skin wounds by using nonviral gene therapy based on minicircle vascular endothelial growth factor DNA and a cationic dendrimer. J. Gene Med., 14: 272-278.

Liu, J.P., J. Baker, A.S. Perkins, E.J. Robertson and A. Efstratiadis, 1993. Mice carrying null mutations of the genes encoding insulinlike growth factor I (Igf-1) and type 1 IGF receptor (Igfr). Cell, 75: 59-72.

Liu, X. and P.X. Ma, 2009. Phase separation, pore structure and properties of nanofibrous gelatin scaffolds. Biomaterials, 30: 4094-4103.

Marchese, C., M. Chedid, O. R. Dirsch, K. G. Csaky, F. Santanelli and C. Latini, 1995. Modulation of keratinocyte growth factor and its receptor in reepithelializing human skin. J. Exp. Med., 182: 1369-1376.

Min, B. M., G. Lee, S. H. Kim, Y. S. Nam, Y. S. Lee and W.H. Park, 2004. Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts *in vitro*. Biomaterials, 25: 1289-1297.

Min, B.M., L. Jeong, K.Y. Lee and W.H. Park, 2006. Regenerated silk fibroin nanofibers: Water vapor-induced structural changes and their effects on the behavior of normal human cells. Macromol. Biosci., 6: 285-292.

Murphy, K. E., C. L. Hall, S. W. McCue and D.S. McElwain, 2011. A two-compartment mechanochemical model of the roles of transforming growth factor â and tissue tension in dermal wound healing. J. Theor. Biol., 272: 145-159.

Namgung, S., K.Y. Baik, J. Park and S. Hong, 2011. Controlling the growth and differentiation of human mesenchymal stem cells by the arrangement of individual carbon nanotubes. ACS Nano, 5: 7383-7390.

Nissen, N. N., R. L. Gamelli, P. J. Polverini and L.A. DiPietro, 2003. Differential angiogenic and proliferative activity of surgical and burn wound fluids. J. Trauma, 54: 1205-1210.

Okamoto, Y., M. Watanabe, K. Miyatake, M. Morimoto, Y. Shigemasa and S. Minami, 2002. Effects of chitin/chitosan and their oligomers/monomers on migrations of fibroblasts and vascular endothelium. Biomaterials, 23: 1975-1979.

Ott, H. C., T. S. Matthiesen, S. K. Goh, L. D. Black and S.M. Kren *et al*., 2008. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat. med., 14: 213-221.

Park, K. E., S. Y. Jung, S. J. Lee, B. M. Min and W.H. Park, 2006. Biomimetic nanofibrous scaffolds: preparation and characterization of chitin/silk fibroin blend nanofibers. Int. j. Biol. Macromol., 38: 165-173.

Petersen, T. H., E. A. Calle, L. Zhao, E. J. Lee and L. Gui *et al*., 2010. Tissue-engineered lungs for *in vivo* implantation. Sci., 329: 358-541.

Quint, C., Y. Kondo, R.J. Manson, J.H. Lawson, A. Dardik and L. E. Niklason, 2011. Decellularized tissue-engineered blood vessel as an arterial conduit. Proc. Nat. Acad. Sci., 108: 9214-9219.

Schmid, P., P. Itin, G. Cherry, C. Bi and D.A. Cox, 1998. Enhanced expression of transforming growth factor-beta type I and type II receptors in wound granulation tissue and hypertrophic scar. Am. J. Pathol., 152: 485-493.

Semenova, E., H. Koegel, S. Hasse, J. E. Klatte and E. Slonimsky *et al*., 2008. Overexpression of mIGF-1 in keratinocytes improves wound healing and accelerates hair follicle formation and cycling in mice. Am. J. Pathol., 173: 1295-1310.

Shah, M., D.M. Foreman and M. Ferguson, 1995. Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. J. Cell Sci., 108: 985-1002.

Shin, H., B.D. Olsen and A. Khademhosseini, 2012. The mechanical properties and cytotoxicity of cell-laden double-network hydrogels based on photocrosslinkable gelatin and gellan gum biomacromolecules. Biomaterials, 33: 3143-3152.

Shin, S. R., H. Bae, J. M. Cha, J. Y. Mun and Y.C. Chen *et al*., 2011. Carbon nanotube reinforced hybrid microgels as scaffold materials for cell encapsulation. ACS nano, 6: 362-372.

Sill, T.J. and H.A. Recum, 2008. Electrospinning: Applications in drug delivery and tissue engineering. J. Biomaterials, 29: 1989-2006.

Slaughter, B. V., S. S. Khurshid, O. Z. Fisher, A. Khademhosseini and N.A. Peppas, 2009. Hydrogels in regenerative medicine. Adv. Mate., 21: 3307-3329.

Spielberger, R., P. Stiff, W. Bensinger, T. Gentile and D. Weisdorf *et al*., 2004. Palifermin for oral mucositis after intensive therapy for hematologic cancers. N. Engl. J. Med., 351: 2590-2598.

Spies, M., O. Nesic, R. E. Barrow, J. R. Perez-Polo and D.N. Herndon, 2001. Liposomal IGF-1 gene transfer modulates pro-and anti-inflammatory cytokine mRNA expression in the burn wound. Gene Ther., 8: 1409-1415.

Tanaka, A., T. Nagate and H. Matsuda, 2005. Acceleration of wound healing by gelatin film dressings with epidermal growth factor. J. Vet. Med. Sci., 67: 909-913.

Thisse, B. and C. Thisse, 2005. Functions and regulations of fibroblast growth factor signaling during embryonic development. Dev. Biol., 287: 390-402.

Thorne, C., 2007. Grabb and Smith's Plastic Surgery. Lippincott Williams and Wilkins, Philadelphia.

Throm, A.M., W.C. Liu, C.H. Lock and K.L. Billiar, 2010. Development of a cell-derived matrix: Effects of epidermal growth factor in chemically defined culture. J. Biomed. Mater. Res. A, 92: 533-541.

Tibbitt, M.W. and K.S. Anseth, 2009. Hydrogels as extracellular matrix mimics for 3D cell culture. Biotechnol. Bioeng., 103: 655-663.

Tomasek, J.J., G. Gabbiani, B. Hinz, C. Chaponnier and R.A. Brown, 2003. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat. Rev. Mol. Cell Biol., 3: 349-363.

Uhl, E., F. Rosken, A. Sirsjo and K. Messmer, 2003. Influence of platelet-derived growth factor on microcirculation during normal and impaired wound healing. Wound Repair Regen., 11: 361-367.

Ulubayram, K., A. N. Cakar, P. Korkusuz, C. Ertan and N. Hasirci, 2001. EGF containing gelatin-based wound dressings. Biomaterials, 22: 1345-1356.

Unemori, E. N., N. Ferrara, E. A. Bauer and E.P. Amento, 2005. Vascular endothelial growth factor induces interstitial collagenase expression in human endothelial cells. J. Cell. Physiol., 153: 557-562.

Uygun, B.E., A. Soto-Gutierrez, H. Yagi, M.L. Izamis and M.A. Guzzardi *et al*., 2010. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat. med., 16: 814-820.

Wang, M., J.H. Yu, D.L. Kaplan and G.C. Rutledge, 2006. Production of submicron diameter silk fibers under benign processing conditions by two-fluid electrospinning. Macromolecules, 39: 1102-1107.

Werner, S., M. Breeden, G. Hubner, D.G. Greenhalgh and M.T. Longaker, 1994. Induction of keratinocyte growth factor expression is reduced and delayed during wound healing in the genetically diabetic mouse. J. Invest. Dermatol., 103: 469-473.

Wise, L. M., M. K. Inder, N. C. Real, G. S. Stuart, S.B. Fleming and A.A. Mercer, 2012. The Vascular Endothelial Growth Factor (VEGF)-E encoded by orf virus regulates keratinocyte proliferation and migration and promotes epidermal regeneration. Cell. Microbiol., 14: 1376-1390.

Wong, V.W., K.C. Rustad, M.G. Galvez, E. Neofytou and J. P. Glotzbach *et al*., 2010. Engineered pullulan-collagen composite dermal hydrogels improve early cutaneous wound healing. Tissue Engin. Part A., 17: 631-644.

Wu, H., T. Suzuki, B. Carey, B. C. Trapnell and F.X. McCormack, 2011. Keratinocyte growth factor augments pulmonary innate immunity through epithelium-driven, GM-CSF-dependent paracrine activation of alveolar macrophages. J. Biol. Chem., 286: 14932-14940.

Yang, Y., T. Xia, F. Chen, W. Wei and C. Liu *et al*. 2011. Electrospun fibers with plasmid bFGF polyplex loadings promote Skin wound healing in diabetic Rats. Molecul. Pharm., 9: 48-58.

Yazbeck, R., G. S. Howarth, L. Borges, M. S. Geier, C.L. Smith, G. P. Davidson and R. N. Butler, 2011. Non-invasive detection of a palifermin-mediated adaptive response following chemotherapy-induced damage to the distal small intestine of rats. Cancer Biol. Ther., 12: 399-406.

Yildirimer, L., N.T.K. Thanh and A.M. Seifalian, 2012. Skin regeneration scaffolds: A multimodal bottom-up approach. Trends Biotechnol., 30: 638-648.

Yildirimer, L., N. T. K. Thanhb, M. Loizidoua and A.M. Seifalian, 2011. Toxicology and clinical potential of nanoparticles. Nano Today, 6: 585-607.

Yoo, C. R., I. S. Yeo, K. E. Park, J. H. Park, S. J. Lee, W.H. Park and B.M. Min, 2008. Effect of chitin/silk fibroin nanofibrous bicomponent structures on interaction with human epidermal keratinocytes. Int. j. Biol. Macromol., 42: 324-334.

You, J.O., M. Rafat, G.J.C. Ye and D.T. Auguste, 2011. Nanoengineering the heart: Conductive scaffolds enhance connexin 43 expression. Nano lett., 11: 3643-3648.

Zhang, P., Z. Hong, T. Yu, X. Chen and X. Jing, 2009. <i> *In vivo* mineralization and osteogenesis of nanocomposite scaffold of poly (lactide-<i> co-glycolide) and hydroxyapatite surface-grafted with poly (l-lactide). Biomaterials, 30: 58-70.

Zhu, J., 2010. Bioactive modification of poly (ethylene glycol) hydrogels for tissue engineering. Biomaterials, 31: 4639-4656.