Growth Factors And Cytokines in Regenerative Medicine

The current advent of regenerative medicine caused huge anticipations in many fields of science, especially in biology and medicine. Regenerative medicine essentially copes with regeneration of cells, which ultimately results in the formation of tissues and organs. Tissue engineering and regenerative medicine are considered as a multidisciplinary field, in which knowledge of pathophysiology of disease, biomaterials, growth factors, and stem cells to repair, replace or regenerate damaged organs and tissues to restore function are combined [1,2]. The inspiration for regenerative medicine strategies commonly is originated from our increasing knowledge regarding how cells and biological systems decrypt cues, and it targets replicating the biological concepts and instructions expressed during embryonic development, such as signal transduction pathways, protein regulation, and transcription factor instructions.

Growth factors and cytokines in Regenerative Medicine

Growth factors (GFs) are natural proteins which play important roles in molecules signaling. They exist in the extracellular matrix (ECM), either soluble in the aqueous environment or bound to other ECM proteins, to bind to receptors on the cell surface to start biological processes [3]. Growth factors can be used to encourage different activities at different stages of regeneration, unlike the tissue engineering materials providing constant support. For instance, for angiogenesis, fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and angiopoietin-2 can be utilized to provide disrupting pre-existing blood vessels and inducing migration to form new ones, then angiopoietin-1 and platelet-derived growth factor BB (PDGF-BB) can be utilized to stabilize recently formed blood vessels [4]. GFs are fundamentally short-lived and lead to very short growth factor half-lives in vivo enzymatic degradation, as short as 3 min for basic fibroblast growth factor (bFGF) [5] and 45 min for nerve growth factor (NGF) [6].

The significance and role of growth factors and cytokines

Growth factors and cytokines with a significant role in regenerative medicine and tissue engineering have generated much interest and numerous clinical trials, however, the results of most of these trials were inacceptable mainly. The specific cellular response initiated by growth factor signaling can lead to a very extensive range of cell actions, such as cell survival, differentiation or proliferation of a specific subset of cells. However, as described above, their bioavailability, lasting the only minute to hours in vivo, provides a hindrance to sustained and controlled delivery. They stimulate cell proliferation, maturation, and differentiation, which are different in growth factors. This complex procedure is regulated by many factors such various cytokines and GFs including platelet-derived growth factor (PDGF), transforming growth factors ? (TGF-?), the bone morphogenetic proteins (BMPs) and the proangiogenic vascular endothelial growth factors (VEGFs), which are the main GFs promoting bone regeneration [7-10]. Therefore, much research is presently focused on engineering growth factors into supportive tissue engineering materials that mimic the natural ECM.

Cytokines are a family of proteins with a low molecular weight that is produced by different cell types. They are responsible for regulating the immune response, inflammation, tissue remodeling and cellular differentiation.

Target cells of growth factors and cytokines include mesenchymal, epithelial and endothelial cells. These molecules normally involve overlapping activities and can act in a paracrine or autocrine mode. Current developments in recombinant protein technologies caused an increase in applying the growth factors as therapeutic agents.

Limitation for their translation to clinical use

- GFs Targets Function
- BMP-2 bone, cartilage bone, differentiation and migration of osteoblasts
- BMP-7 cartilage, kidney differentiation and migration of osteoblasts, renal development
- PDGF-AB (or -BB) blood vessel, muscle, bone, cartilage, skin embryonic development, proliferation, migration, growth of endothelial cells
- TGF-a brain, skin proliferation of basal cells or neural cells
- TGF-b bone, cartilage proliferation and differentiation of bone-forming cells, anti-proliferative factor for epithelial cells VEGF blood vessel migration, proliferation and survival of endothelial cells
- Limitation for their translation to clinical use

Most of the growth factors and cytokines that entered in clinical trials failed or raised main safety concerns when being approved [11-13]. For instance, PDGF-BB has entered in clinical practice for treating chronic wounds [14], however, its effectiveness is not considerable and it received boxed warnings from the US Food and Drug Administration (USFDA) concerning elevated cancer risk [15]. No controlled delivery system exists in PDGF-BB. Therefore, the side effects probably are originated from GF uncontrolled diffusion to the surrounding cells and tissues (Fig. 1a). Other growth factors were discovered for skin healing with even less success including transforming growth factor-?3 (TGF-?3) [16- 18]. Regarding bone healing, it was believed that bone morphogenetic protein-2 (BMP-2) are perfect to induce bone formation with no adverse events, however, nowadays, a long list of safety and cost-effectiveness issues with BMP-2 [19] exists. As an ultimate instance, interleukin (IL)-1 receptor antagonist (IL-1Ra) that in our study is considered as a cytokine, is used for curing rheumatoid arthritis [14]. However, several local and systemic side effects are encouraged by IL-1Ra [1]. In clinical trials and commercial products, growth factors and cytokines are utilized at extremely high doses with no appropriate delivery systems.

The rapid breakdown and clearance of GFs from tissue sites in vivo are among the causes of this poor clinical translation. To provide retention of GFs within matrixes, controlled-release and protein engineering strategies have been explored.

Definitely, supra-physiological levels of these molecules coupled with poor delivery kinetics are the main reasons for the various side effects, low effectiveness, and extra costs. Therefore, considering safety risks and poor cost-effectiveness, there is a strong need to develop better delivery systems (Fig. 1b).

Hypothesis and aims: We hypothesize that engineering growth factors and cytokines to bind ECM components very strongly (i.e. conferring super-affinity) using an ECM-binding domain derived from placenta growth factor-2 (PlGF-2) will drastically improve their delivery and regenerative potential. The new generation of growth factors and cytokines will have a built-in delivery system allowing them to stay in the tissue where they are delivered and they will be effective at doses where their wild-type forms are not (Fig. 1a, b). Thus, we seek to engineer

IL-1Ra to target two applications, namely chronic skin wound repair and bone regeneration. In the context of skin, we will also engineer two distinct proteins to prevent scarring from surgical incisions, namely TGF-?3 and IL-10 (Fig. 1c).

Growth factor interactions with synthetic biomaterials and natural ECM

Strategies for biomaterial presentation of growth factors

In tissue engineering, two distinct approaches involve for delivery of growth factors: (i) chemical immobilization of the growth factor into or onto the matrix which includes binding affinity interaction between the growth factor with polymer substrate and a cell or a tissue and (ii) physical encapsulation of growth factors in the delivery system (Fig. 2). The second approach is obtained by the encapsulation, diffusion and pre-programmed release of growth factor from the substrate into the surrounding tissue.

Growth factor interaction with ECM proteins

Recent researches attempting to develop better biomimetic strategies have focused on the natural binding interactions between GFs and their environments. Physiologically, most growth factors and cytokines bind the ECM, which critically controls their bioavailability and presentation to cells. In translating growth factors and cytokines, this fundamental aspect has been overlooked. So, engineering growth factors and cytokines to bind ECM components very strongly by using ECM-binding domain will drastically improve their delivery and regenerative potential.

1- Biomaterials engineered with GFs-binding domains: Thanks to heparan sulphate, Many GFs are known to bind ECM proteins within the matrix. Mimicking these natural interactions, biomaterials have been engineered with heparin (mimicking heparan sulfates) to immobilized heparin-binding GFs [23, 24]. Via GF-binding sites contained inside ECM proteins, some natural interactions also exist between ECM proteins (e.g. fibrinogen, fibronectin) and GFs. Therefore, the functionalization of biomaterials with GF-binding domains (such as the heparin-binding domain of fibrinogen or the 12th-14th type III repeats of fibronectin), makes the creation of microenvironments possible that are able to interact with GFs specifically and control their delivery at the target site [25-28] (Fig. 3A).

2- GFs engineered with ECM binding domains: Other approaches rather than engineering the biomaterials, have focused on engineering GFs themselves, using domains capable of binding ECM proteins (Fig. 3B). Nevertheless, this principle appears to provide the main advantage which is the possible GF delivery without any biomaterials usages. In some specific cases, to pass clinical regulations and trials, non-existence of the biomaterials compounds in the delivery system could be the main advantage.

In the recent researches, engineering GFs with ECM-binding domains derived from GFs has emphasized. For instance, in a study which has conducted by Martino, et al., different growth factors have tested for binding to key ECM molecules commonly present in tissues (fibronectin, vitronectin, osteopontin, collagen I, and fibrinogen). This experiment revealed that PlGF-2 is able to bind ECM proteins strongly while PlGF-1 is not able to bind them (Fig. 4). By analyzing

and comparing these two sequences they indicated that the ECM-binding domain is within PlGF-2123-144. This domain was found to be responsible for the binding characteristics of PlGF-2, by fusing the sequence (RRRPKGRGKRRREKQRPTDCHL) to a model protein (glutathione-sulfotransferase (GST)) which is unable to bind ECM proteins.

Moreover, they tested the super- affinity of fused growth factor for ECM proteins and found that PlGF-2123-144 insertion into growth factors provide super-affinity for ECM proteins and heparan sulfate, however, they did not change their ability to activate their receptors. Dissociation constant (KD) values of the PlGF-2123-144–fused growth factors for ECM proteins were provided to similar values as those exhibited by PlGF-2, resulting in 2- to 100-fold elevations in affinity, in comparison with the wild-type growth factors (table.2). Furthermore, PlGF-2123-144–fused growth factors could be retained in fibrin (mimicking a clot) or in tissues strongly, where they were injected, demonstrating binding to endogenous ECM [29].

Using rational protein engineering, this domain was incorporated to others GFs (VEGF-A165, BMP-2, and PDGF-BB), to create GFs with super-affinity for the ECM. This super-affinity to diverse ECM proteins made the controlled delivery of the engineered GFs possible directly at the injury site with no exogenous biomaterial usage. The simultaneous topical delivery of low doses (40 to 250-time lower than the quantity required for the wild-type GFs) of PlGF-2123-144 fused-PDGF-BB and VEGF-A165 was indicated to improve wound healing and angiogenesis in diabetic mice while decreasing the vascular permeability. Furthermore, it was proved that the topical delivery of PlGF-2123-144 fused-PDGF-BB and BMP-2 at low doses is capable of improving bone regeneration in a rat model.