A novel human TGF-β1 fusion protein and type I collagen serve as a scaffold for adult mesenchymal stem cells in bone regeneration

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The regulation of stem cell functions including cell adhesion and migration, mitosis and differentiation into specific tissue cells is now considered a crucial strategy to improve the regenerative potential of scaffolding matrices used for tissue engineering [1,2]. The stem cell niche, which is a pool of microenvironmental cues that control the fate of stem cells, is highly specified depending on the tissue types in terms of physical, chemical and mechanical characteristics [3]. Therefore, providing a physiological situation that mimics the native tissue environment may switch on the action of stem cells to enter into an appropriate stage and undergo tissue differentiation [4,5].

Mesenchymal stem cells (MSCs) are known to have multipotency to develop into a series of cell lineages, including osteoblast, adipocyte, chondrocyte and neural cells, in response to appropriate chemical and physical cues [6,7]. When compared with embryonic stem cells, MSCs are accessible without the possible concerns regarding ethical issues and can be readily obtained from adults, allowing their clinical application [8,9]. Recent studies have demonstrated the potential usefulness of MSCs for the treatment of defective and diseased tissues, including bone, cartilage and nerves [9]. Above all, the importance of microenvironmental control has been highlighted to enable complete use of MSCs in tissue engineering and regenerative therapy [10,11].

Transforming growth factor-betas (TGF-βs) are a superfamily of molecules closely related in structure and function. It is now well established that members of the TGF-β family play a prominent role in the development, growth and maintenance of the vertebrate skeleton [12,13]. The effect of TGF-β1 on the proliferation and osteoblastic differentiation of MSCs in vitro –causing an increase in total cell number, alkaline phosphatase (ALP) activity, and osteocalcin (OC) production- is well documented [14,15].

Collagen gel has been a model for culturing cells in three-dimensional environments, which is a condition much more similar to native tissue extracellular matrix than two-dimensional culture dishes [16,17]. Specifically, the collagen fibrous network and surrounding medium fluid constitute a soft and flexible gel matrix that allows cells to freely reach out and migrate in three dimensions [1,18]. Many groups have used collagen gel matrix to investigate the behavior and differentiation of MSCs into specific cell lineages, such as chondrocytes, osteoblasts and endothelial cells [2,17].

To define the responses of mesenchymal osteoprogenitor stem cells to TGF-β1, we cultured Fischer 344 rat bone marrow (BM) cells in a collagen gel medium containing 0.5% fetal bovine serum for prolonged periods of time. Under these conditions, survival of BM MSCs was dependent on the addition of TGF-β1. Recombinant human TGF-β1-F2 (rhTGF-β1-F2), a fusion protein engineered to contain an auxiliary collagen binding domain (von Willebrand's factor-derived), demonstrated the ability to support survival colony formation and growth of the surviving cells, whereas commercial TGF-β1 did not. Initially, cells were selected from a whole BM cell population and captured inside a collagen network, on

the basis of their survival response to added exogenous growth factors. After the 10-day selection period, the surviving cells in the rhTGF-β1-F2 test groups proliferated rapidly in response to serum factors (10% FBS), and maximal DNA synthesis levels were observed (Fig. 1). Upon the addition of osteoinductive factors, osteogenic differentiation *in vitro* was evaluated by the induction of ALP expression (Fig. 2), and the formation of mineralized matrix (data not shown).

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Figures

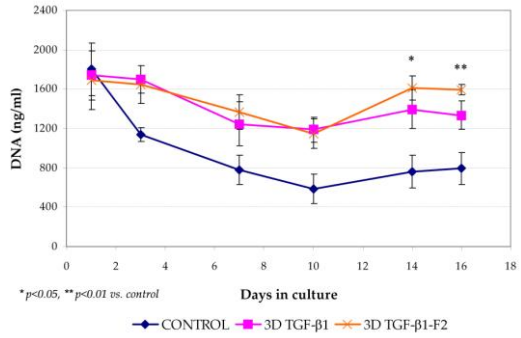

Figure 1. Quantification of DNA content as indication of cell replication.

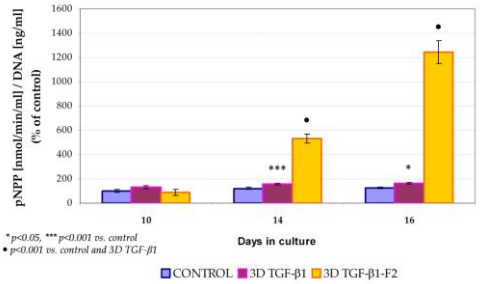

Figure 2. Effects of culture conditions on ALP activity.