Influence of a Growth Factor-Protein Mixture on the Development of the Osteogenic Phenotype on Titanium

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Statement of Purpose: Growth factors (GFs), a class of signaling proteins that stimulate cell proliferation and/or differentiation in target cells, have been used in a series of strategies to promote tissue healing, usually in conjunction with delivery systems to control the speed, time and quantity of release. Nevertheless, the use of platelet-rich plasma (PRP) for bone repair applications has been questioned in terms of its efficacy to increase new bone formation. The present study aimed to evaluate the effect of a 10-fold serial dilution (1:1, 1:10, and 1:100) of a PRP-like growth factor-protein mixture on key parameters of the development of the osteogenic phenotype on titanium (Ti).

Methods: The mixture of GFs and proteins tested was selected from major components found in porcine[1] and human[2] platelet preparations and contained 27 ng/mL rhPDGF-BB, 22 ng/mL rhTGF-β1, 15 ng/mL rhTGF-β2, 3.7 µg/mL serum-derived human albumin, 2 µg/mL plasma-derived human fibronectin, and 0.5 µg/mL platelet-derived thrombospondin (Sigma) diluted in osteogenic medium. Osteoblastic cells were obtained by enzymatic digestion of newborn rat calvarial bone and plated on polished, machined Ti discs (13 mm in diameter). During the first 7 days of culture, calvarial cells were exposed to three different dilutions of GFs + proteins mixture (1:1, 1:10 and 1:100, also referred to as GFs + proteins, GFs + proteins/10, and GFs + proteins/100, respectively). Because many of the PRP effects on cells do not reflect simple combination of its major GFs, cells were also exposed to the mixture containing only the protein constituents. Control cultures were only exposed to the osteogenic medium. The parameters evaluated were: 1) cell viability by MTT assay at day 7; 2) Immunolabeling to detect bone sialoprotein (BSP), an early marker of osteoblast differentiation, at days 7 and 14; 3) Alkaline phophatase (ALP) activity and total protein content at days 7 and 10, and 4) mineralized bone-like nodule formation at day 14. Comparisons were carried out using the non-parametrical Kruskal-Wallis test, followed by the Fischer test based on rank, where appropriate. The level of significance was set at 5%.

Results: Cell viability assay at day 7 revealed that mitochondrial activity was significantly higher for GFs + proteins cultures, decreasing to control levels for GFs + proteins/10 and GFs + proteins/100 groups. At the same time point, control cultures exhibited large areas of BSP positive cells, mostly in sites of initial cell multilayering, whereas treated cultures showed only focal sites of weak BSP labeling. At day 7, there was a significant difference in ALP activity between the control and the GFs + proteins treated groups, which exhibited low levels. At day 10, ALP specific activity increased in all experimental groups, except for GFs + proteins, which remained unaltered, but there was no significant GFs + proteins dose-response relationship. The highest ALP activity was observed in the control, followed by the proteins only group. Total protein content performed at days 7 and 10 correlated with the MTT results at day 7, with significantly greater values for GFs + proteins cultures. At day 14, qualitative and quantitative analyses revealed that control, proteins and GFs + proteins/100 groups supported the development of mineralized bone-like nodule formation (Fig. 1); nodules stained with Alizarin red and were BSP immunoreactive. Proportions of Alizarin red stained areas ranked as follows: Control = Proteins > GFs + proteins/100 > GFs + proteins/10 = GFs + proteins. Strikingly, a complete lack of bone-like nodule formation was noticed for GFs + proteins and GFs + proteins/10 treated cultures. Data are presented in Table 1.

Table 1. Quantitative analysis (mean ± SD) of MTT assay (nm), alkaline phosphatase (ALP) activity (µmol thymolphthalein/h/mg protein), total protein content (µg/mL), and proportion of calcified areas (%) of control, GFs + proteins, GFs + proteins/10, GFs + proteins/100, and proteins treated rat calvarial osteogenic cell cultures grown on Ti discs

	MTT assay	ALP activity		Total protein content		Mineralization
	Day 7	Day 7	Day 10	Day 7	Day 10	Day 14
Control	0.12 ± 0.01	6.2 ± 2.6	24.9 ± 5.4	58.0 ± 3.2	92.6 ± 3.4	56.6 ± 10.1
GFs+proteins	0.20 ± 0.02	2.2 ± 1.4	2.0 ± 0.6	81.3 ± 7.4	99.0 ± 10.1	0.9 ± 0.3
GFs+proteins/10	0.13 ± 0.01	1.0 ± 0.4	5.0 ± 1.9	67.5 ± 4.9	96.0 ± 9.1	0.7 ± 0.7
GFs+proteins/100	0.10 ± 0.01	0.4 ± 0.9	3.4 ± 2.0	46.5 ± 3.1	73.9 ± 5.8	26.4 ± 1.4
Proteins	0.12 ± 0.02	1.2 ± 0.9	8.6 ± 2.1	53.9 ± 3.5	87.0 ± 11.4	46.8 ± 0.7

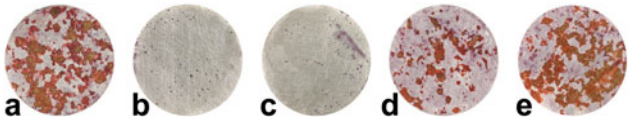

Figure 1. Macroscopic imaging of Alizarin red staining for (a) control, (b) GFs+proteins, (c) GFs+proteins/10, (d) GFs+proteins/100, and (e) proteins treated osteogenic cell cultures grown on Ti, at day 14.

Conclusion: The results of the present study show that a GFs + proteins mixture, which contains the major components of PRP preparations, inhibits the progression of osteoblastic phenotype of rat calvarial cells grown on Ti. In addition, dilution of the mixture can eventually partially restore bone-like nodule formation.

References: 1. Venne et al. J Neuroradiol 1999;26:92-100. **2.** Martineau et al. Biomaterials 2004;25:4489-4502.

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