In Vitro Osteoblastic Differentiation on Bioactive Glass and Glass-ceramic Surfaces

Alves OC[1], de Oliveira FS[1], Fernandes RR[1], Zanotto ED[2], Peitl O[2], Beloti MM[1], Rosa AL[1], de Oliveira PT[1]

1 Ribeirao Preto Dental School, University of Sao Paulo, Ribeirao Preto (SP), Brazil
2 Federal University of Sao Carlos, Sao Carlos (SP), Brazil

Statement of Purpose: Bioactive glasses and glass-ceramics have been used as bone substitutes in either particulate or scaffold forms. Various thermal treatments that allow the development of scaffolds from bioactive glasses may create varied proportions of new crystalline phases in the amorphous phase with a potential impact on the bioactivity and biocompatibility of the material. The aim of the present *in vitro* study was to qualitatively and quantitatively evaluate the development of the osteogenic phenotype in osteoblastic cell cultures grown on bioactive glass and glass-ceramic surfaces.

Methods: MC3T3-E1 cells, subclone 14, were cultured under an osteogenic condition for periods up to 21 days on the following disc surfaces: Bioglass® 45S5 (bioactive glass), Biosilicate® (bioactive glass-ceramic), Biosilicate® as the material for scaffold preparation (Bio-sc, bioactive glass-ceramic), and borosilicate (bioinert glass). At days 7, 12 and 21 post-plating, cell morphology, mineralized matrix formation and the expression profile of genes associated with osteogenesis were evaluated.

Results: The quantitative analysis of Alizarin red-stained cultures at day 21 revealed significantly enhanced mineralization in cultures grown on bioactive materials compared with the ones on borosilicate and the highest absorbance intensities for the Bio-sc group (Figure 1). Differential gene expression profiles were detected at the three time points evaluated in cultures grown on the bioactive materials in comparison with borosilicate (Table 1).

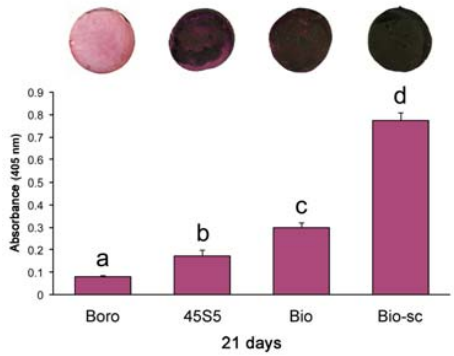

Figure 1. Macroscopic aspects of cultured MC3T3-E1 cells on borosilicate (Boro), Bioglass ® 45S5 (45S5) Biosilicato ® (Bio) and scaffold for Biosilicato ® (Bio-sc), stained with Alizarin red (ARS), and quantifying ARS, indicating the greatest osteogenic potential of crops on Bio-sc. Bars with different letters indicate significant differences between groups.

Table 1. Proportions of genes up- and down regulated in MC3T3-E1 cells grown on the different groups for periods of 7, 12 and 21 days compared to those on borosilicate. Dark shades represent over expression (green) or repression (red) in a proportion greater than or equal to 50% of the genes evaluated in each group, whereas those in light shades, less than 50%

Period/Groups of genes	Day 7			Day 12			Day 21		
	45S5	Bio	Bio-sc	45S5	Bio	Bio-sc	45S5	Bio	Bio-sc
Up regulated									
Bone matrix proteins	4/5	3/5	3/5	1/5	3/5	2/5	2/5	2/5	2/5
BMPs superfamily	5/10	5/10	0/10	1/10	3/10	2/10	6/10	2/10	3/10
Receptors	10/16	5/16	4/16	0/16	9/16	4/16	12/16	6/16	10/16
Growth factors	4/10	1/10	0/10	1/10	6/10	4/10	6/10	2/10	3/10
Integrin receptors	5/6	3/6	0/6	0/6	3/6	4/6	5/6	3/6	5/6
Collagen	6/14	7/14	3/14	2/14	8/14	5/14	10/14	6/14	10/14
Cartilage related	1/2	0/2	0/2	0/2	0/2	0/2	1/2	1/2	1/2
Metalloproteinases	1/4	0/4	0/4	0/4	3/4	2/4	2/4	1/4	2/4
Transcription factors	2/8	1/8	0/8	0/8	3/8	4/8	7/8	5/8	8/8
Other genes	2/9	2/9	0/9	0/9	2/9	3/9	4/9	2/9	3/9
Down regulated									
Bone matrix proteins	0/5	0/5	1/5	2/5	0/5	0/5	0/5	0/5	0/5
BMPs superfamily	0/10	1/10	1/10	2/10	0/10	0/10	0/10	0/10	0/10
Receptors	1/16	3/16	2/16	4/16	1/16	0/16	0/16	0/16	0/16
Growth factors	0/10	2/10	1/10	2/10	1/10	1/10	0/10	0/10	0/10
Integrin receptors	0/6	0/6	0/6	1/6	0/6	1/6	0/6	0/6	0/6
Collagen	1/14	5/14	3/14	7/14	0/14	4/14	0/14	1/14	0/14
Cartilage related	0/2	1/2	0/2	2/2	0/2	0/2	0/2	0/2	0/2
Metalloproteinases	1/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
Transcription factors	0/8	4/8	0/8	3/8	0/8	0/8	0/8	0/8	0/8
Other genes	0/9	1/9	1/9	1/9	0/9	0/9	0/9	1/9	0/9

Conclusions: From the results presented, it can be concluded that changes in chemical characteristics of glass and glass-ceramic that may have an impact on their bioactivity index can affect the osteogenic potential and the gene expression profile of osteoblastic cells *in vitro*. The highest osteogenic activity on Bio-sc renders this material a good candidate for bone defect applications.

References:

CHEN QZ, Biomaterials 2006; 27:2414-2425.
BELLUCCI D, Sci Sinter 2010; 42:307-320.
JAMES PF. J Non-Cryst Solids 1995; 181:1-15.

Supported by the State of Sao Paulo Research Foundation (FAPESP), Brazil.