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Thorne et al.

(54) **COMPOSITION AND PROCESS FOR BONE GROWTH AND REPAIR**

(76) Inventors: **Kevin J. Thorne**, Arvada, CO (US); **James J. Benedict**, Arvada, CO (US)

Correspondence Address:
Timothy L. Scott, Esq.
Sulzer Biologics Inc.
Suite 1600
3 East Greenway Plaza
Houston, TX 77046-0391 (US)

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(57) **ABSTRACT**

A composition for the induction of bone growth is disclosed. The composition includes a substrate, bone growth protein, and sources of calcium and phosphate. The composition is acidic which promotes high activity of the bone growth protein. The calcium and phosphate sources can be provided as an acidic calcium phosphate salt. Also disclosed are methods of the making the composition and methods of using it.

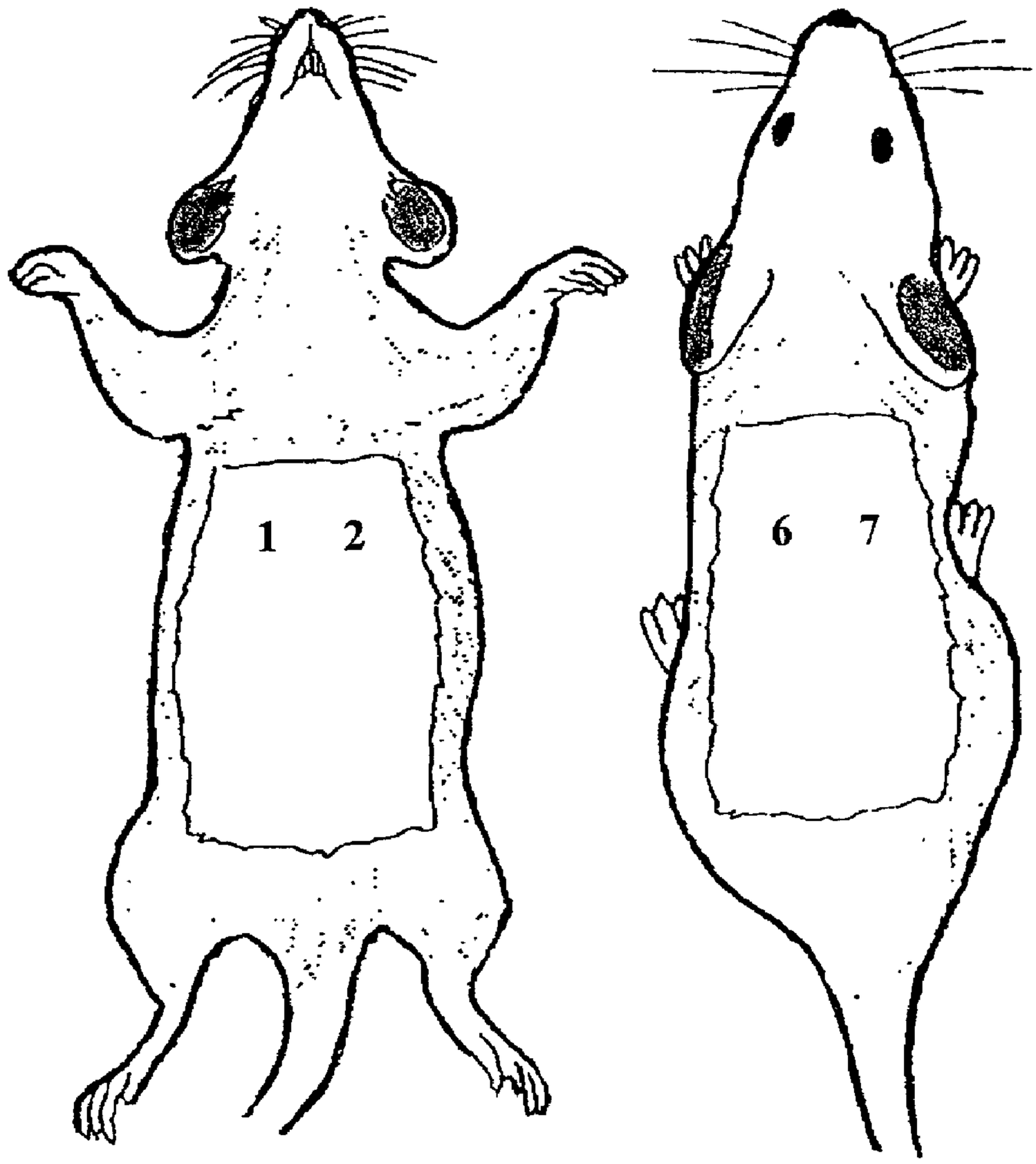

Figure 1

Figure 2 A

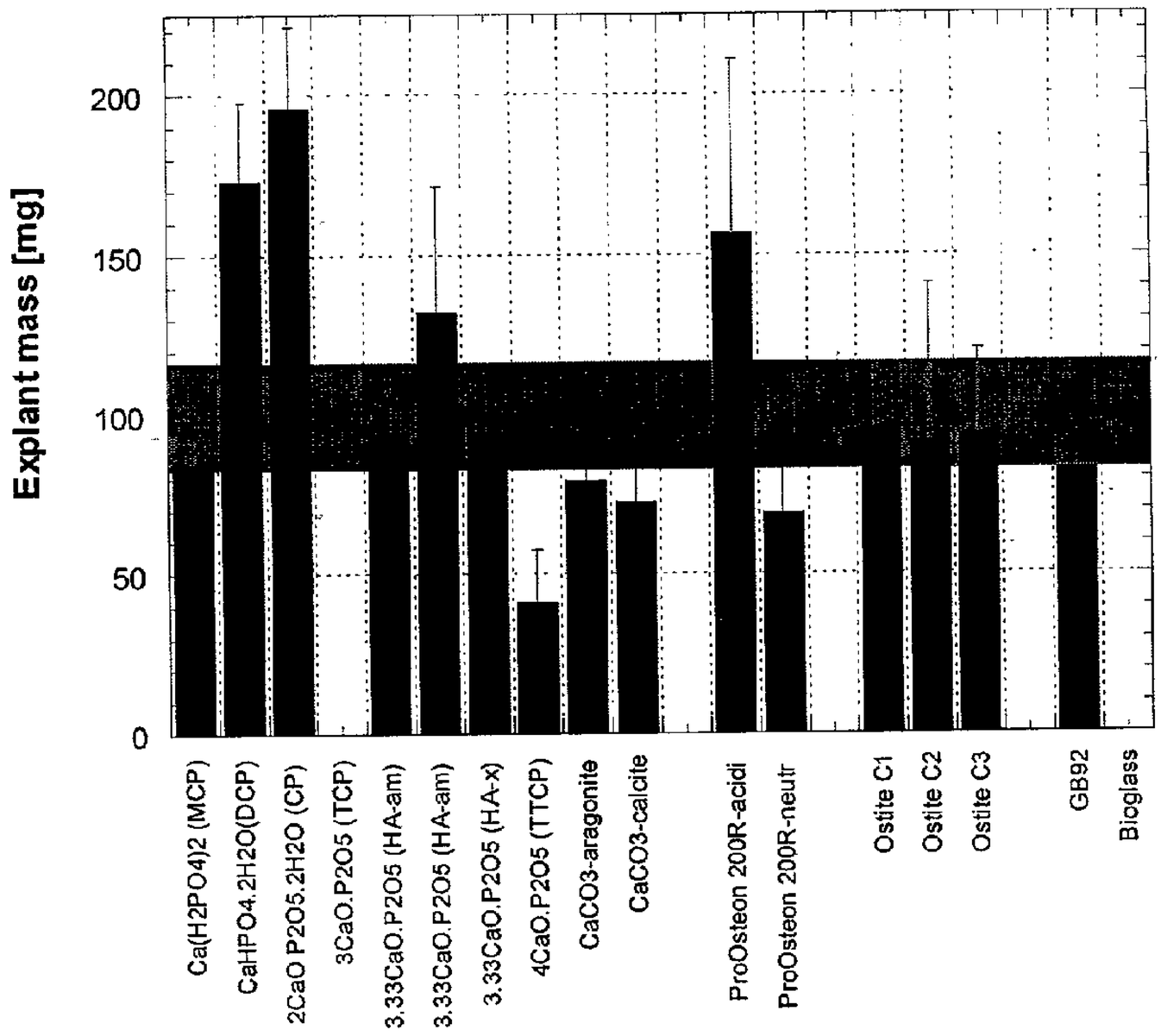

Figure 2 B

	Explant Mass				Explant Mass - *normalized*			
	CPB		CB		CPB		CB	
	avg	*±SD*	avg	*±SD*	avg	*±SD*	avg	*±SD*
$Ca(H_2PO_4)_2$ (MCP)	71.2	*17.0*	84.8	*11.0*	84.7	*20.2*	100.8	*15.5*
$CaHPO4.2H_2O$(DCP)	157.2	*22.4*	91.6	*10.6*	173.0	*24.7*	100.8	*15.5*
$2CaO.P_2O_5.2H2O$ (CP)	191.8	*24.6*	98.6	*13.7*	196.1	*25.2*	100.8	*15.5*
$3CaO.P_2O_5$ (TCP)								
$3.33CaO.P_2O_5$ (HA-am)	134.8	*15.2*	144.8	*29.8*	93.9	*10.6*	100.8	*15.5*
$3.33CaO.P_2O_5$ (HA-am)	136.2	*39.9*	103.8	*13.4*	132.3	*38.8*	100.8	*15.5*
$3.33CaO.P_2O_5$ (HA-x)	109.0	*18.1*	116.2	*8.6*	94.6	*15.7*	100.8	*15.5*
$4CaO.P_2O_5$ (TTCP)	46.8	*18.8*	115.0	*5.9*	41.0	*16.5*	100.8	*15.5*
$CaCO_3$-aragonite	96.6	*41.0*	123.2	*15.1*	79.1	*33.6*	100.8	*15.5*
$CaCO_3$-calcite	73.4	*24.1*	102.2	*17.6*	72.4	*23.8*	100.8	*15.5*
ProOsteon 200R-acidi	87.5	*30.2*	56.3	*16.3*	156.7	*54.1*	100.8	*15.5*
ProOsteon 200R-neutr	22.0	*4.9*	32.2	*6.5*	68.9	*15.3*	100.8	*15.5*
								0.0
Ostite C1	108.3	*13.4*	114.2	*13.8*	95.6	*11.8*	100.8	*15.5*
Ostite C2	101.6	*55.0*	112.2	*22.8*	91.3	*49.4*	100.8	*15.5*
Ostite C3	109.8	*31.5*	118.6	*26.3*	93.3	*26.8*	100.8	*15.5*
GB9N	80.8	*10.8*	98.6	*13.4*	82.6	*11.0*	100.8	*15.5*
Bioglass								

Figure 3 A

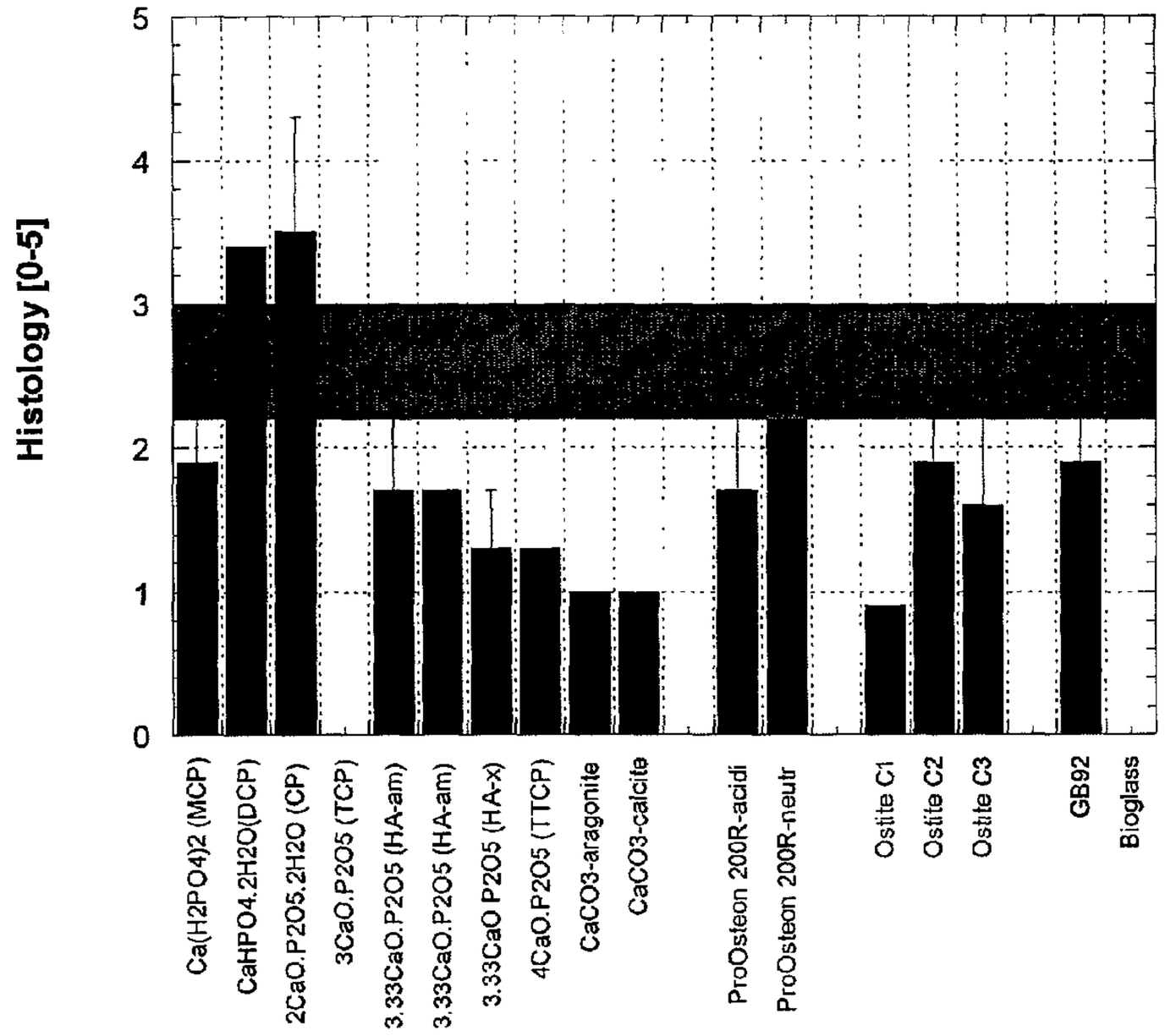

Figure 3 B

	Histology Score				Histology Score - *normalized*			
	CPB		CB		CPB		CB	
	avg	*±SD*	avg	*±SD*	avg	*±SD*	avg	*±SD*
$Ca(H_2PO_4)_2$ (MCP)	1.7	*0.6*	2.3	*0.6*	1.9	*0.7*	2.6	*0.4*
$CaHPO4.2H_2O$(DCP)	3.0	*0.0*	2.3	*0.6*	3.4	*0.0*	2.6	*0.4*
$2CaO.P_2O_5.2H2O$ (CP)	2.7	*0.6*	2.0	*0.0*	3.5	*0.8*	2.6	*0.4*
$3CaO.P_2O_5$ (TCP)								
$3.33CaO.P_2O_5$ (HA-am)	1.3	*0.6*	2.0	*0.0*	1.7	*0.8*	2.6	*0.4*
$3.33CaO.P_2O_5$ (HA-am)	2.0	*0.0*	3.0	*0.0*	1.7	*0.0*	2.6	*0.4*
$3.33CaO.P_2O_5$ (HA-x)	1.2	*0.4*	2.4	*0.5*	1.3	*0.4*	2.6	*0.4*
$4CaO.P_2O_5$ (TTCP)	1.0	*0.0*	2.0	*0.0*	1.3	*0.0*	2.6	*0.4*
$CaCO_3$-aragonite	1.0	*0.0*	2.7	*0.6*	1.0	*0.0*	2.6	*0.4*
$CaCO_3$-calcite	1.0	*0.0*	2.7	*0.6*	1.0	*0.0*	2.6	*0.4*
ProOsteon 200R-acidi	2.8	*1.0*	4.4	*0.5*	1.7	*0.6*	2.6	*0.4*
ProOsteon 200R-neutr	2.0	*0.0*	2.4	*0.5*	2.2	*0.0*	2.6	*0.4*
								0.0
Ostite C1	1.0	*0.0*	3.0	*0.0*	0.9	*0.0*	2.6	*0.4*
Ostite C2	1.7	*0.6*	2.3	*0.6*	1.9	*0.7*	2.6	*0.4*
Ostite C3	1.7	*1.2*	2.7	*0.6*	1.6	*1.2*	2.6	*0.4*
GB9N	2.0	*1.0*	2.7	*0.6*	1.9	*1.0*	2.6	*0.4*
Bioglass								

Figure 4 A

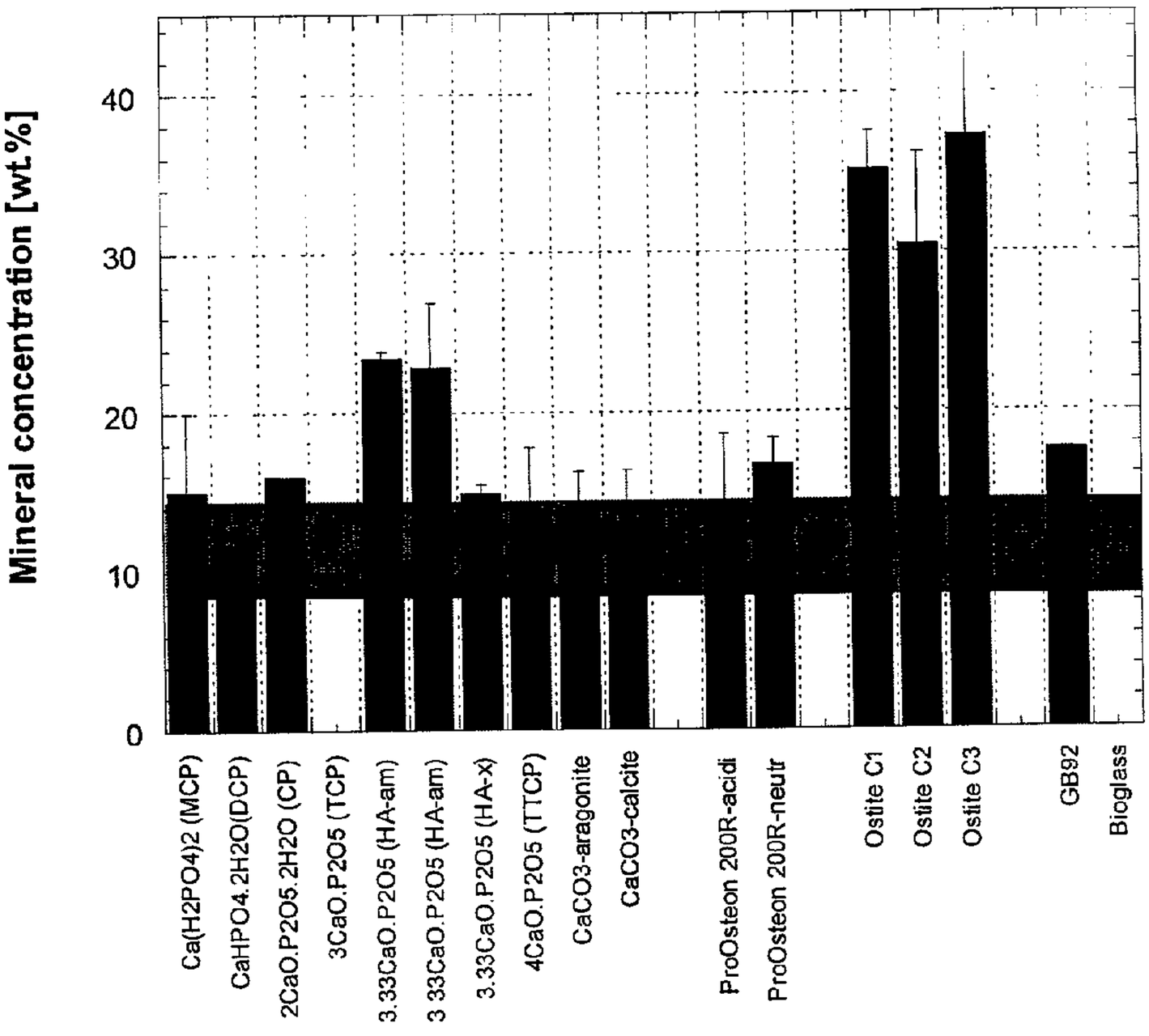

Figure 4 B

	Mineral Concentration				Mineral conc - *normalized*			
	CPB		CB		CPB		CB	
	avg	*±SD*	avg	*±SD*	avg	*SD*	avg	*±SD*
$Ca(H_2PO_4)_2$ (MCP)	12.3	*4.1*	8.8	*1.9*	15.0	*5.0*	10.7	*1.4*
$CaHPO4.2H_2O$(DCP)	12.1	*0.5*	11.1	*0.7*	11.7	*0.5*	10.7	*1.4*
$2CaO.P_2O_5.2H2O$ (CP)	16.4	*0.0*	11.1	*0.5*	15.9	*0.0*	10.7	*1.4*
$3CaO.P_2O_5$ (TCP)								
$3.33CaO.P_2O_5$ (HA-am)	18.2	*0.3*	8.3	*1.8*	23.5	*0.4*	10.7	*1.4*
$3.33CaO.P_2O_5$ (HA-am)	18.1	*3.2*	8.5	*1.4*	22.9	*4.0*	10.7	*1.4*
$3.33CaO.P_2O_5$ (HA-x)	18.5	*0.6*	13.3	*1.4*	14.9	*0.5*	10.7	*1.4*
$4CaO.P_2O_5$ (TTCP)	17.8	*5.0*	13.7	*0.9*	13.9	*3.9*	10.7	*1.4*
$CaCO_3$-aragonite	14.6	*2.3*	11.2	*1.6*	14.0	*2.2*	10.7	*1.4*
$CaCO_3$-calcite	12.2	*3.0*	10.0	*1.6*	13.1	*3.2*	10.7	*1.4*
ProOsteon 200R-acidi	19.3	*7.2*	15.3	*4.2*	13.5	*5.1*	10.7	*1.4*
ProOsteon 200R-neutr	25.4	*2.8*	16.4	*3.2*	16.6	*1.8*	10.7	*1.4*
Ostite C1	26.6	*1.8*	8.1	*0.7*	35.2	*2.4*	10.7	*1.4*
Ostite C2	23.6	*4.5*	8.3	*0.5*	30.5	*5.8*	10.7	*1.4*
Ostite C3	25.7	*5.7*	7.4	*0.5*	37.3	*8.3*	10.7	*1.4*
GB9N	15.6	*0.1*	9.5	*0.8*	17.6	*0.1*	10.7	*1.4*
Bioglass								

Figure 5 A

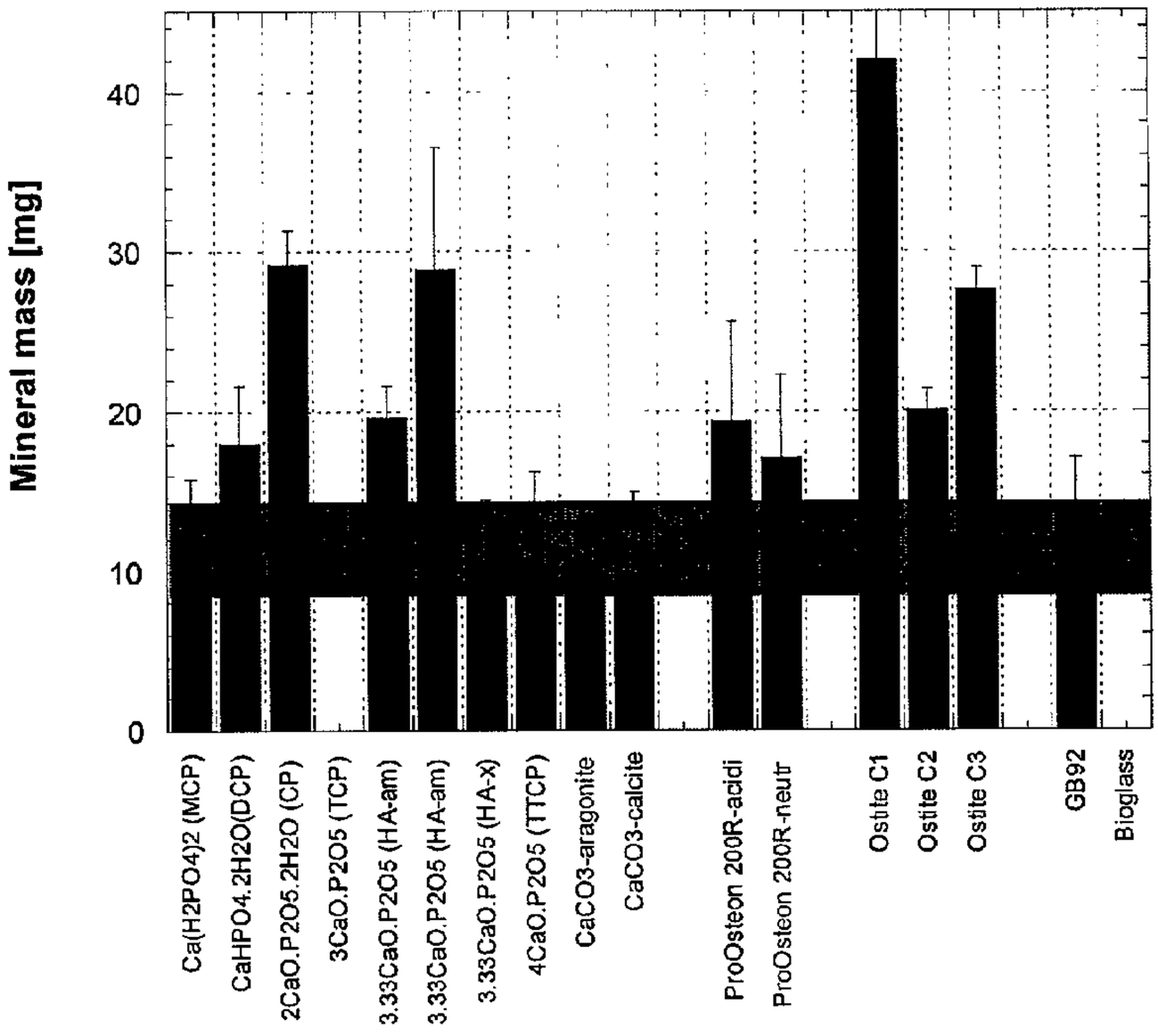

Figure 5 B

	Mineral Mass				Mineral Mass - *normalized*			
	CPB		CB		CPB		CB	
	avg	*±SD*	avg	*±SD*	avg	*±SD*	avg	*±SD*
$Ca(H_2PO_4)_2$ (MCP)	9.9	*3.0*	8.5	*0.7*	12.1	*3.7*	10.4	*1.8*
$CaHPO4.2H_2O$(DCP)	18.2	*3.6*	10.5	*2.2*	18.0	*3.6*	10.4	*1.8*
$2CaO.P_2O_5.2H2O$ (CP)	33.3	*2.4*	11.8	*0.8*	29.2	*2.1*	10.4	*1.8*
$3CaO.P_2O_5$ (TCP)								
$3.33CaO.P_2O_5$ (HA-am)	26.8	*2.8*	14.2	*5.1*	19.6	*2.0*	10.4	*1.8*
$3.33CaO.P_2O_5$ (HA-am)	26.8	*7.0*	9.6	*1.9*	28.9	*7.6*	10.4	*1.8*
$3.33CaO.P_2O_5$ (HA-x)	16.7	*6.4*	16.4	*0.3*	10.5	*4.0*	10.4	*1.8*
$4CaO.P_2O_5$ (TTCP)	14.3	*7.8*	14.1	*1.6*	10.5	*5.7*	10.4	*1.8*
$CaCO_3$-aragonite	13.2	*4.3*	14.5	*2.7*	9.4	*3.1*	10.4	*1.8*
$CaCO_3$-calcite	8.2	*4.2*	8.6	*1.8*	9.9	*5.1*	10.4	*1.8*
ProOsteon 200R-acidi	10.3	*3.3*	5.5	*1.6*	19.4	*6.2*	10.4	*1.8*
ProOsteon 200R-neutr	6.6	*2.0*	4.0	*1.8*	17.1	*5.2*	10.4	*1.8*
Ostite C1	34.5	*5.7*	8.5	*1.3*	42.0	*6.9*	10.4	*1.8*
Ostite C2	20.8	*1.3*	10.7	*0.1*	20.1	*1.3*	10.4	*1.8*
Ostite C3	25.8	*1.3*	9.7	*1.6*	27.6	*1.4*	10.4	*1.8*
GB9N	11.8	*2.7*	8.8	*0.4*	13.9	*3.2*	10.4	*1.8*
Bioglass								

Figure 6 A

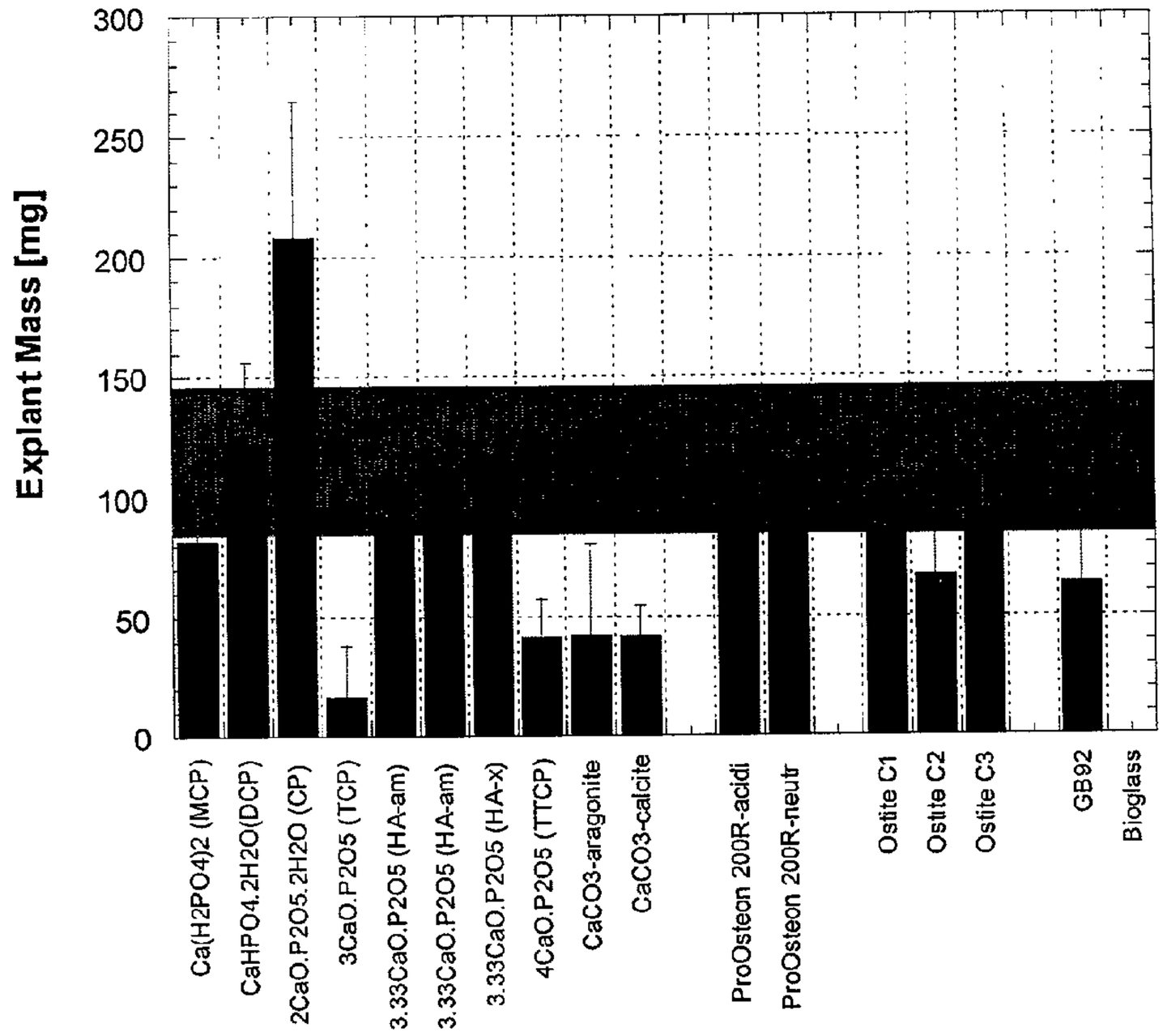

Figure 6 B

	Explant Mass				Explant Mass - *normalized*			
	CPB		CDB		CPB		CDB	
	avg	*±SD*	avg	*±SD*	avg	*±SD*	avg	*±SD*
$Ca(H_2PO_4)_2$ (MCP)	107.5	*22.5*	154.7	*24.3*	81.6	*17.1*	117.5	*28.9*
$CaHPO4.2H_2O$(DCP)	78.9	*21.6*	75.5	*16.9*	122.7	*33.6*	117.5	*28.9*
$2CaO.P_2O_5.2H2O$ (CP)	98.9	*27.0*	55.8	*36.1*	208.2	*56.8*	117.5	*28.9*
$3CaO.P_2O_5$ (TCP)	11.7	*15.3*	83.2	*20.7*	16.5	*21.6*	117.5	*28.9*
$3.33CaO.P_2O_5$ (HA-am)	107.3	*29.4*	137.4	*15.3*	91.7	*25.1*	117.5	*28.9*
$3.33CaO.P_2O_5$ (HA-am)	124.6	*25.6*	130.2	*29.4*	112.4	*23.1*	117.5	*28.9*
$3.33CaO.P_2O_5$ (HA-x)	111.1	*16.5*	110.7	*25.6*	117.9	*17.5*	117.5	*28.9*
$4CaO.P_2O_5$ (TTCP)	41.1	*16.0*	116.9	*16.0*	41.3	*16.1*	117.5	*28.9*
$CaCO_3$-aragonite	50.1	*45.1*	139.3	*45.1*	42.2	*38.0*	117.5	*28.9*
$CaCO_3$-calcite	68.0	*21.0*	191.0	*21.0*	41.8	*12.9*	117.5	*28.9*
ProOsteon 200R-acidi	74.7	*13.6*	80.4	*29.1*	109.1	*21.4*	117.5	*28.9*
ProOsteon 200R-neutr	29.2	*6.0*	35.5	*12.7*	96.6	*24.1*	117.5	*28.9*
								0.0
Ostite C1	123.0	*46.3*	167.3	*28.5*	86.3	*4.2*	117.5	*28.9*
Ostite C2	82.9	*21.2*	146.3	*28.6*	66.6	*37.2*	117.5	*28.9*
Ostite C3	127.0	*26.6*	157.5	*31.2*	94.7	*15.8*	117.5	*28.9*
GB9N	52.8	*20.5*	97.5	*22.6*	63.6	*24.7*	117.5	*28.9*
Bioglass								

Figure 7 A

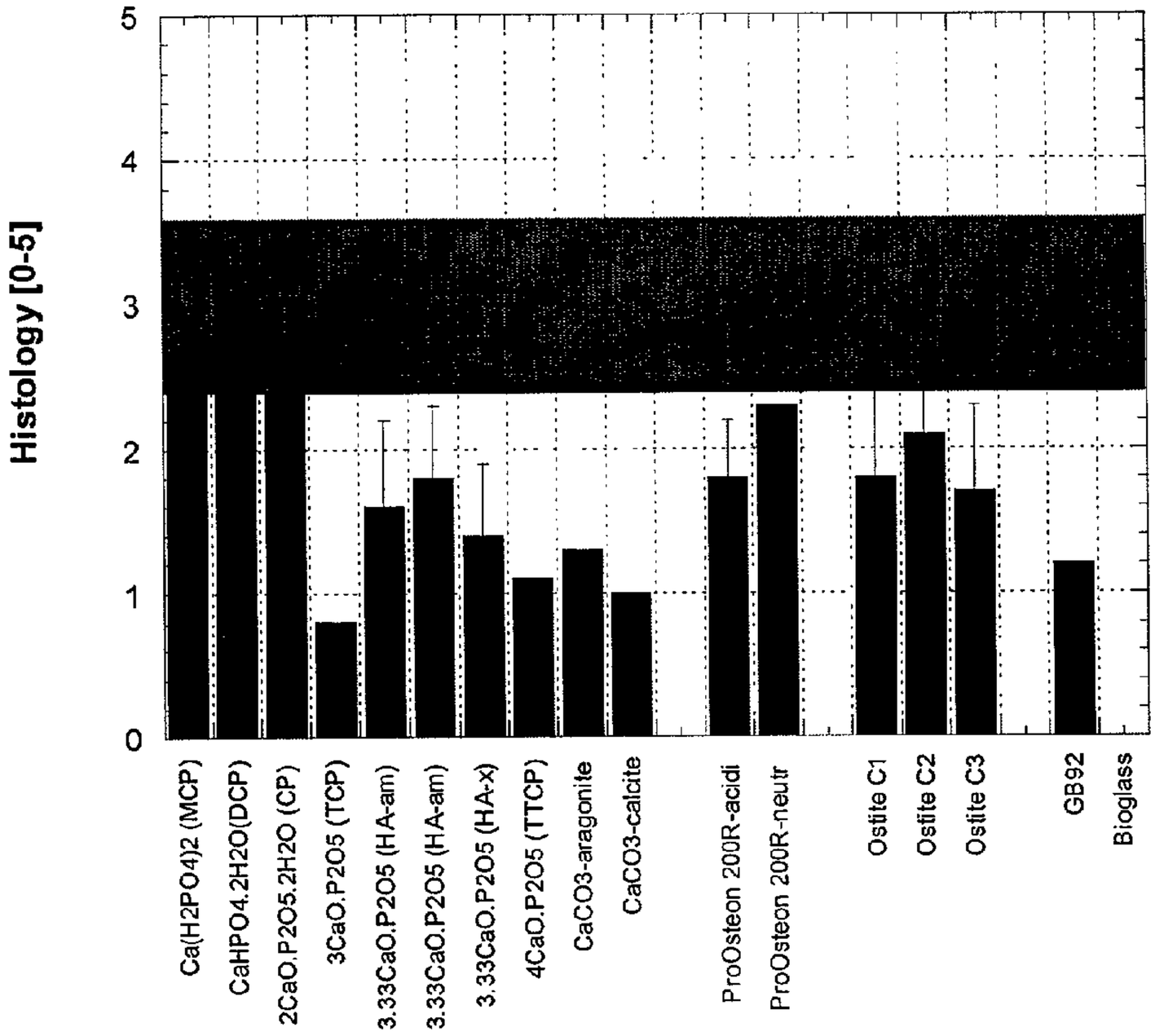

Figure 7 B

	Histology Score				Histology Score - *normalized*			
	CPB		CDB		CPB		CDB	
	avg	*±SD*	avg	*±SD*	avg	*±SD*	avg	*±SD*
$Ca(H_2PO_4)_2$ (MCP)	3.0	*0.0*	3.7	*0.5*	2.4	*0.0*	3.0	*0.6*
$CaHPO4.2H_2O$(DCP)	4.0	*0.6*	3.8	*0.6*	3.1	*0.5*	3.0	*0.6*
$2CaO.P_2O_5.2H2O$ (CP)	2.3	*0.8*	2.8	*1.3*	2.4	*0.8*	3.0	*0.6*
$3CaO.P_2O_5$ (TCP)	1.0	*0.0*	3.7	*0.5*	0.8	*0.0*	3.0	*0.6*
$3.33CaO.P_2O_5$ (HA-am)	1.3	*0.5*	2.4	*0.7*	1.6	*0.6*	3.0	*0.6*
$3.33CaO.P_2O_5$ (HA-am)	1.1	*0.3*	1.8	*0.6*	1.8	*0.5*	3.0	*0.6*
$3.33CaO.P_2O_5$ (HA-x)	1.3	*0.5*	2.7	*0.5*	1.4	*0.5*	3.0	*0.6*
$4CaO.P_2O_5$ (TTCP)	1.0	*0.0*	2.7	*0.5*	1.1	*0.0*	3.0	*0.6*
$CaCO_3$-aragonite	1.0	*0.0*	2.3	*0.6*	1.3	*0.0*	3.0	*0.6*
$CaCO_3$-calcite	1.0	*0.0*	3.0	*0.0*	1.0	*0.0*	3.0	*0.6*
ProOsteon 200R-acidi	2.5	*0.6*	4.0	*0.0*	1.8	*0.4*	3.0	*0.6*
ProOsteon 200R-neutr	2.0	*0.0*	2.6	*0.9*	2.3	*0.0*	3.0	*0.6*
								0.0
Ostite C1	1.9	*0.8*	3.1	*0.4*	1.8	*0.8*	3.0	*0.6*
Ostite C2	2.1	*0.8*	2.9	*0.3*	2.1	*0.8*	3.0	*0.6*
Ostite C3	1.8	*0.6*	3.2	*0.4*	1.7	*0.6*	3.0	*0.6*
GB9N	1.0	*0.0*	2.5	*0.6*	1.2	*0.0*	3.0	*0.6*
Bioglass								

Figure 8 A

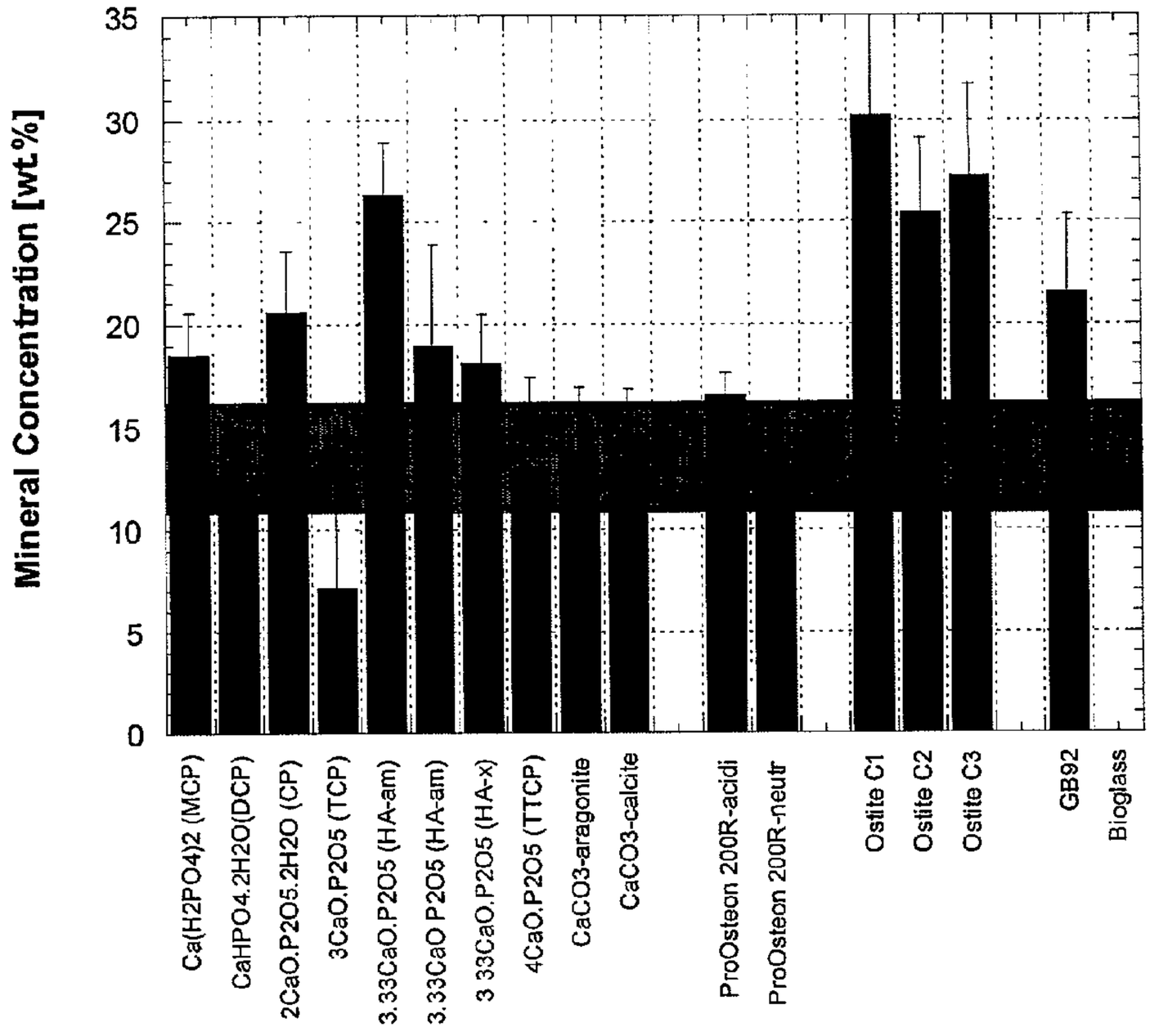

Figure 8 B

	Mineral Concentration				Mineral conc. - *normalized*			
	CPB		CDB		CPB		CDB	
	avg	*±SD*	avg	*±SD*	avg	*±SD*	avg	*±SD*
$Ca(H_2PO_4)_2$ (MCP)	18.0	*2.0*	13.1	*1.8*	18.5	*2.1*	13.5	*2.7*
$CaHPO4.2H_2O$(DCP)	11.9	*2.7*	13.0	*2.1*	12.3	*2.8*	13.5	*2.7*
$2CaO.P_2O_5.2H2O$ (CP)	17.3	*2.5*	11.3	*1.0*	20.6	*3.0*	13.5	*2.7*
$3CaO.P_2O_5$ (TCP)	7.6	*7.0*	14.4	*1.8*	7.1	*6.6*	13.5	*2.7*
$3.33CaO.P_2O_5$ (HA-am)	24.6	*2.4*	12.6	*2.1*	26.3	*2.6*	13.5	*2.7*
$3.33CaO.P_2O_5$ (HA-am)	18.3	*4.7*	13.0	*1.4*	19.0	*4.9*	13.5	*2.7*
$3.33CaO.P_2O_5$ (HA-x)	18.9	*2.5*	14.1	*2.6*	18.1	*2.4*	13.5	*2.7*
$4CaO.P_2O_5$ (TTCP)	13.1	*4.4*	13.6	*2.1*	13.0	*4.4*	13.5	*2.7*
$CaCO_3$-aragonite	14.5	*3.3*	14.2	*4.0*	13.8	*3.1*	13.5	*2.7*
$CaCO_3$-calcite	12.3	*3.0*	12.3	*3.0*	13.5	*3.3*	13.5	*2.7*
ProOsteon 200R-acidic	19.1	*1.3*	15.6	*2.6*	16.5	*1.1*	13.5	*2.7*
ProOsteon 200R-neutral	22.6	*2.8*	23.2	*15.2*	13.1	*1.6*	13.5	*2.7*
Ostite C1	22.2	*5.4*	9.9	*2.1*	30.2	*7.3*	13.5	*2.7*
Ostite C2	22.6	*3.3*	12.0	*2.8*	25.4	*3.7*	13.5	*2.7*
Ostite C3	23.2	*3.8*	11.5	*2.8*	27.2	*4.5*	13.5	*2.7*
GB9N Bioglass	18.7	*3.3*	11.7	*0.8*	21.5	*3.8*	13.5	*2.7*

Figure 9 A

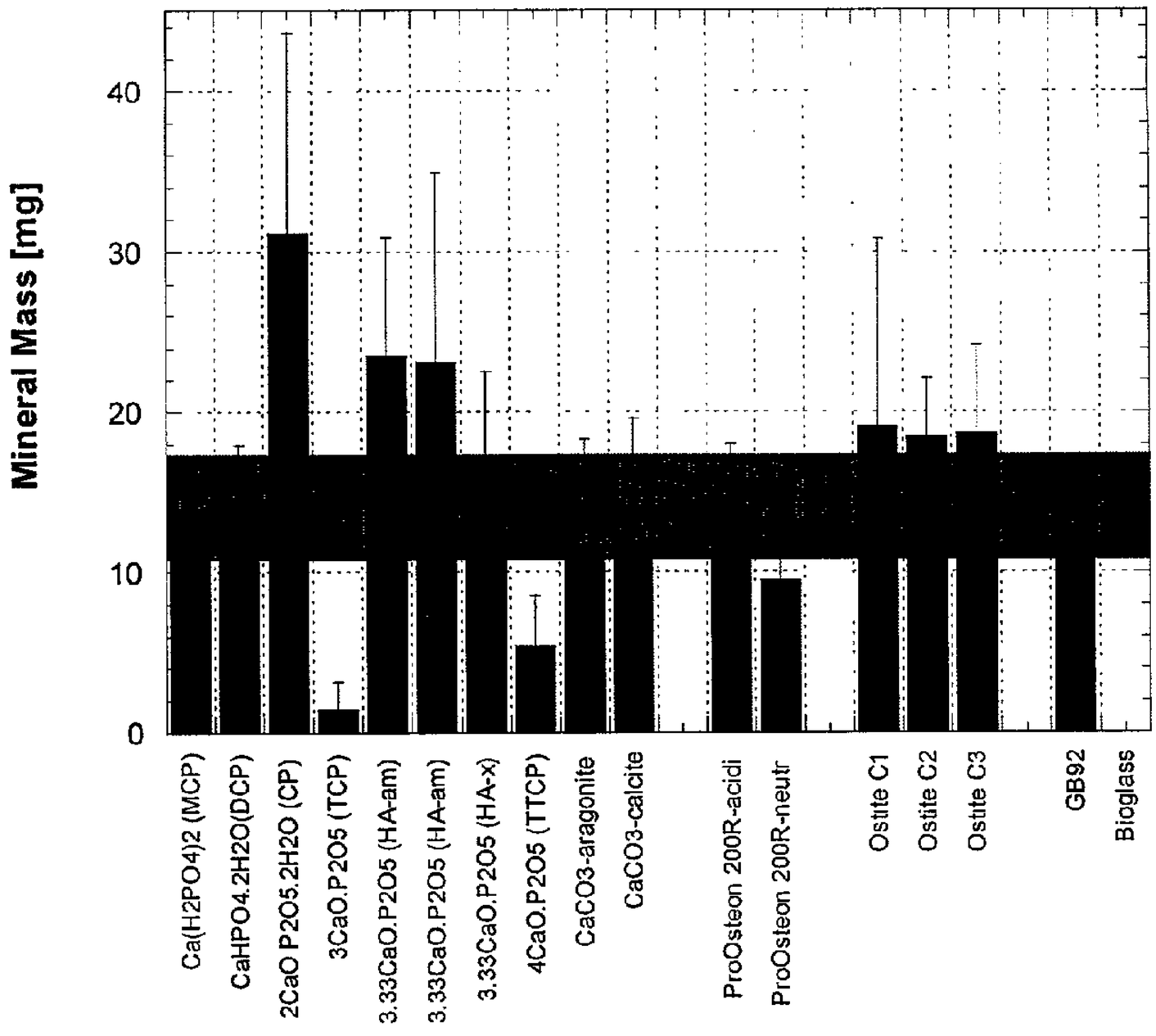

Figure 9 B

	Mineral Mass				Mineral Mass - *normalized*			
	CPB		CDB		CPB		CDB	
	avg	*±SD*	avg	*±SD*	avg	*±SD*	avg	*±SD*
$Ca(H_2PO_4)_2$ (MCP)	17.3	*2.6*	18.4	*3.1*	13.9	*2.1*	14.8	*3.5*
$CaHPO4.2H_2O$(DCP)	9.4	*2.2*	9.6	*3.2*	14.5	*3.4*	14.8	*3.5*
$2CaO.P_2O_5.2H2O$ (CP)	13.3	*5.3*	6.3	*1.1*	31.2	*12.4*	14.8	*3.5*
$3CaO.P_2O_5$ (TCP)	1.1	*1.3*	11.2	*1.6*	1.5	*1.7*	14.8	*3.5*
$3.33CaO.P_2O_5$ (HA-am)	26.2	*8.3*	16.5	*2.8*	23.5	*7.4*	14.8	*3.5*
$3.33CaO.P_2O_5$ (HA-am)	28.9	*14.7*	18.5	*3.3*	23.1	*11.8*	14.8	*3.5*
$3.33CaO.P_2O_5$ (HA-x)	17.7	*6.4*	15.9	*0.9*	16.5	*6.0*	14.8	*3.5*
$4CaO.P_2O_5$ (TTCP)	5.8	*3.4*	16.0	*3.9*	5.4	*3.1*	14.8	*3.5*
$CaCO_3$-aragonite	12.1	*7.2*	15.6	*3.5*	11.5	*6.8*	14.8	*3.5*
$CaCO_3$-calcite	14.2	*9.2*	17.7	*8.5*	11.9	*7.7*	14.8	*3.5*
ProOsteon 200R-acidi	14.7	*4.3*	15.6	*7.0*	13.9	*4.1*	14.8	*3.5*
ProOsteon 200R-neutr	5.9	*1.6*	9.2	*4.4*	9.5	*2.6*	14.8	*3.5*
Ostite C1	22.9	*14.0*	17.7	*2.8*	19.1	*11.7*	14.8	*3.5*
Ostite C2	17.3	*3.5*	13.9	*3.4*	18.4	*3.7*	14.8	*3.5*
Ostite C3	26.1	*7.6*	20.6	*2.8*	18.7	*5.5*	14.8	*3.5*
GB9N Bioglass	10.9	*4.9*	14.0	*2.8*	11.5	*5.2*	14.8	*3.5*

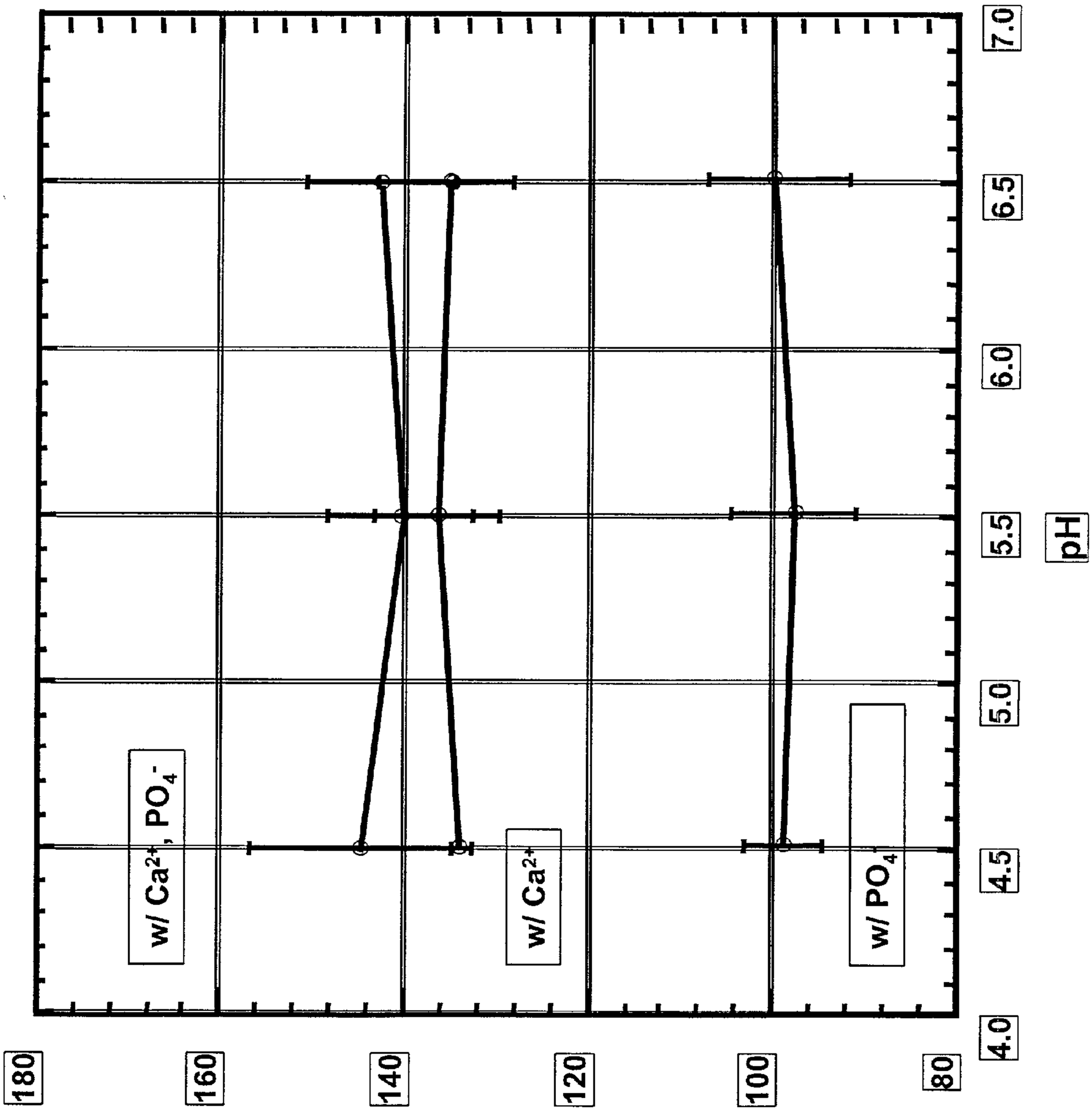
Relative Histology Score [%]
180
160
140
120
100
80
pH
7.0
6.5
6.0
5.5
5.0
4.5
4.0
w/ Ca2+, PO4-
w/ Ca2+
w/ PO4

Figure 10

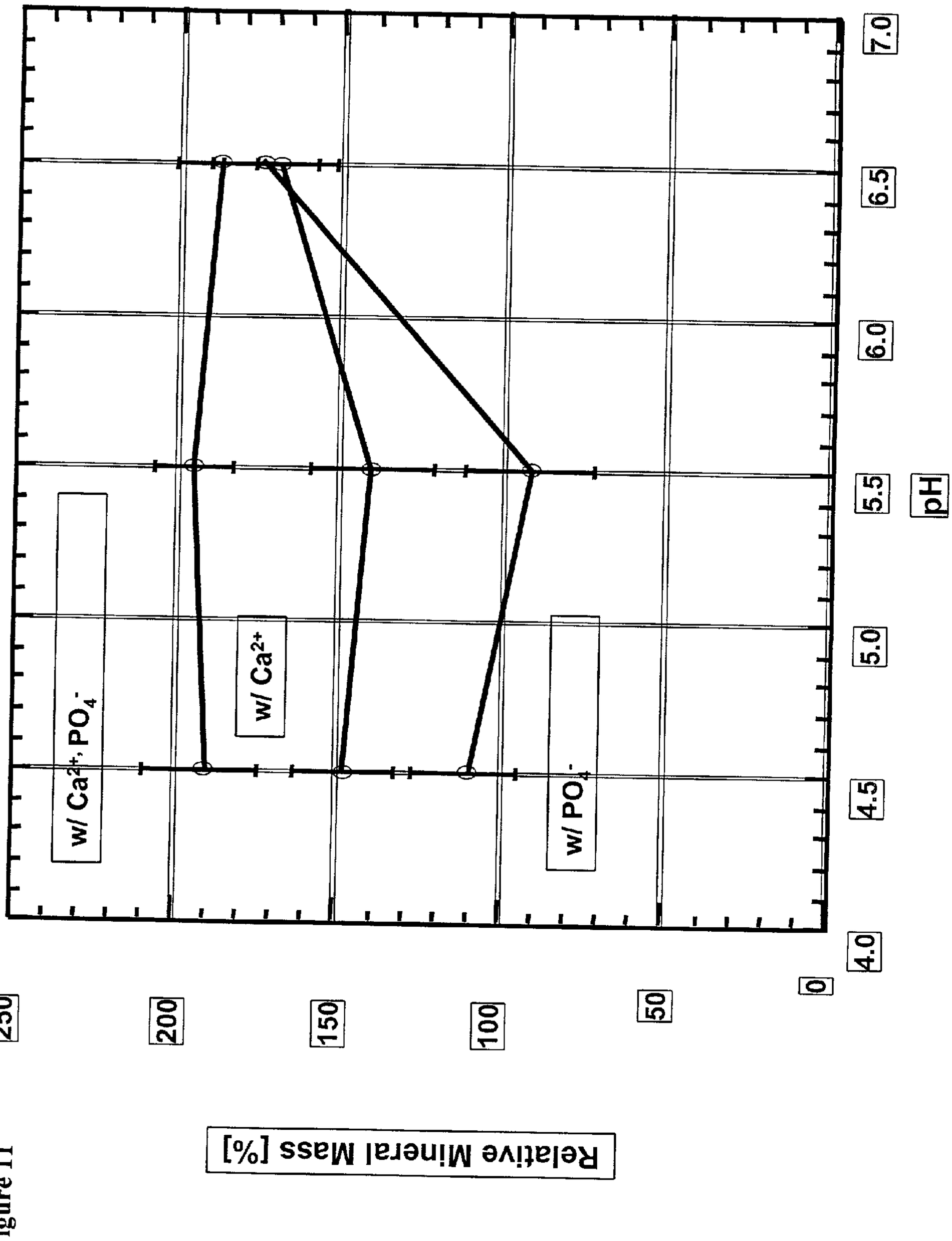
pH
7.0
6.5
6.0
5.5
5.0
4.5
4.0
Relative Mineral Mass [%]
250
200
150
100
50
0
w/ Ca2+, PO4-
w/ Ca2+
w/ PO4-

Figure 11

COMPOSITION AND PROCESS FOR BONE GROWTH AND REPAIR

FIELD OF THE INVENTION

[0001] The present invention relates to methods and compositions for the induction of bone growth in mammals and to methods for the production of such compositions.

BACKGROUND

[0002] A number of diseases or injuries involving bones are known for which regeneration of bone is a desired treatment. Formation of bone in vivo involves an interaction of various inductive proteins which act by causing a differentiation of mesenchymal cells into cartilage and then bone-forming cell lines. This mechanism is not completely understood. However, in efforts to improve orthopedic procedures, purified protein mixtures and recombinantly produced proteins have been developed which stimulate osteoinductive activity.

[0003] In general, autogeneous bone grafts have been viewed as the standard for restoring skeletal defects. However, autogeneous sources of bone in human beings are limited, expensive and painful to obtain. Accordingly, materials such as demineralized bone matrix have been developed to augment or replace autogeneous bone grafts. However, a synthetic alternative to demineralized bone matrix is desired to improve the ease of use, economy of product manufacture and to eliminate the potential of transgenic disease transfer or immune system incompatibilities. To date however, an acceptable synthetic substitute has not been identified.

[0004] Currently the clinical potential of composite implants containing a mixture of bovine tendon collagen and a proprietary bone morphogenic protein mixture, with demineralized bone matrix powders and simulated body fluid is being evaluated. While a number of advances have improved the activity of osteogenic factors such as those present in bone morphogenic protein, their clinical application has been limited by the requirement for a superior delivery vehicle. European resistance to the use of demineralized bone matrix, as well as the need to enhance the activity of the bone morphogenic protein mixture to reduce cost, speaks to the need to develop synthetic substitutes for demineralized bone matrix.

[0005] The present invention provides material compositions that can be used as synthetic alternatives for demineralized bone matrix to obtain a product with an improved osteoinductive response for growth factors in degradable implants for skeletal regeneration. The material compositions of the present invention are easier to use and more economical to manufacture than demineralized bone matrix, and they eliminate the potential of both disease and pathogen transfer and immune system incompatibilities.

[0006] Numerous synthetic materials have been experimentally evaluated as alternative delivery vehicles for osteogenic growth factors. The materials previously assessed by reconstructive surgeons and scientists includes hydroxyapatites, tricalcium phosphates, aliphatic polyesters, cancellous bone allografts, human fibrin, plaster of paris, apatite wollastonite glass ceramics, titanium, devitalized bone matrix, non-collagenous proteins, collagen and autolyzed antigen extracted allogenic bone. None of these materials have been found to be entirely satisfactory.

[0007] Other growth factor carriers containing calcium phosphate additives have been developed. For example, a macroporous collagen sponge containing a mixture of α-tricalcium phosphate (α-3CaO P_2O_5) and hydroxyapatite (3.33CaO $P_2O_5(OH)_2$) has been developed. Alternatively, a macroporous collagen sponge that contains precipitated hydroxyapatite has also been disclosed (U.S. Pat. No. 5,776,193). These products are consistent with the prevailing view that hydroxyapatite is the preferred synthetic for bone graft extenders due to its compositional similarity with the mineral component of natural bone. Although evidence suggests that hydroxyapatite does provide benefits related to osteoblast adherence, the present inventors have now shown that the addition of hydroxyapatite to collagen actually hinders the osteogenic activity of bone morphogenic protein in rats.

[0008] There remains a need for improved compositions for the induction of bone growth in animals that address the problems of existing compositions and products.

SUMMARY

[0009] The present invention is directed to a bone growth composition which includes a substrate, bone growth protein, a source of calcium and a source of phosphate. The composition has an acidic buffering potential in physiological solution. In one embodiment, the composition further includes a biocompatible buffering agent to maintain the acidity of the composition. In further alternative embodiments, the sources of calcium and/or phosphate can be salts such as calcium monophosphate, calcium hydrogen phosphate, or calcium pyrophosphate. The substrate in the composition can be collagen, fibrin, alginate or mixtures thereof. The bone growth protein can be purified bone growth proteins, recombinantly produced bone growth or mixtures thereof. In a preferred embodiment, the bone growth protein includes a purified bone growth protein composition known as Bone Protein.

[0010] The present invention also includes a process for producing an implantable bone growth composition. The process includes producing a dispersion of collagen fibrils containing a solubilized sodium phosphate salt. The process further includes adding a calcium chloride salt to the dispersion of collagen fibrils to precipitate a calcium phosphate salt onto the surface of the collagen fibrils to produce an implantable bone growth composition. Alternatively, the process can include making the dispersion with a calcium salt and adding a phosphate salt. For example, the solubilized sodium phosphate salt can be calcium hydrogen phosphate dihydrate and the calcium phosphate salt can be calcium dichloride dihydrate.

[0011] The present invention also includes a process for the induction of bone formation in a mammal, which includes implanting a bone growth composition of the present invention in a mammal. Such a process can be a hip replacement operation, a knee replacement operation, a spinal fusion, repair of periodontal defects, treatment of osteoporosis, repair of bone defects or repair of bone fractures.

[0012] The composition of the present invention and products made therewith are superior synthetic materials for use

as a demineralized bone matrix replacement. It has been found that the calcium source, the phosphate source and the acidic buffering potential each have independent beneficial effects for bone growth induced by the present composition. In addition, the novel processing technology for producing such materials produces collagen sponges with dramatically superior physical properties. The products are collagen dispersions containing a calcium phosphate salt on the surface of the collagen fibrils, resulting in the formation of water stable, collagen sponges with superior physical properties. Composite products provide both improved physical properties and superior osteogenic performance. The products can be quickly and cheaplyprepared, and can reduce the required doses of bone morphogenic proteins. These composites provide significant economic savings and eliminate potential allograft disease transfer due to the elimination of demineralized bone matirx. Additionally, the composites provide reduced bone morphogenic protein dosing, provide more reproducible clinical results, allow simpler, cheaper surgical application and better maintain physical dimensions during vertebral fusion.

BRIEF DESCRIPTION OF THE DRAWINGS

[**0013**] **FIG. 1**: Indicates location of the subcutaneous implant sites in the upper quadrants of a rat's abdomen and dorsal thorax.

[**0014**] **FIG. 2**: A. Explant mass of disks composed of osteogenic compounds at time of harvest, normalized to average value measured against controls containing only collagen and bone proteins.

[**0015**] B. Average and normalized values of explant mass at time of harvest.

[**0016**] **FIG. 3**: A. Histology scores of disks composed of osteogenic compounds at time of harvest normalized to average value measured against controls containing only collagen and bone proteins.

[**0017**] B. Average and normalized histology scores at time of harvest.

[**0018**] **FIG. 4**: A. Mineral concentration of disks composed of osteogenic compounds at time of harvest normalized to average value measured against controls containing only collagen and bone proteins.

[**0019**] B. Average and normalized mineral concentration at time of harvest.

[**0020**] **FIG. 5**: A. Mineral mass of disks composed of osteogenic compounds at time of harvest normalized to average value measured against controls containing only collagen and bone proteins.

[**0021**] B. Average and normalized mineral mass at time of harvest.

[**0022**] **FIG. 6**: A. Explant mass of disks composed of osteogenic compounds at time of harvest normalized to average value measured against controls containing collagen, bone proteins and devitalized bone matirx.

[**0023**] B. Average and normalized values of explant mass at time of harvest.

[**0024**] **FIG. 7**: A. Histology scores of disks composed of osteogenic compounds at time of harvest normalized to average value measured against controls containing collagen, bone proteins and devitalized bone matirx.

[**0025**] B. Average and normalized histology scores at time of harvest.

[**0026**] **FIG. 8**: A. Mineral concentration of disks composed of osteogenic compounds at time of harvest normalized to average value measured against controls containing collagen, bone proteins and devitalized bone matirx.

[**0027**] B. Average and normalized mineral concentration at time of harvest.

[**0028**] **FIG. 9**: A. Mineral mass of disks composed of osteogenic compounds at time of harvest normalized to average value measured against controls containing collagen, bone proteins and devitalized bone matirx.

[**0029**] B. Average and normalized mineral mass at time of harvest.

[**0030**] **FIG. 10**: shows the influence of adding a calcium source, a phosphate source, or both a calcium and a phosphate source to the implanted composition at different acidic buffering capacity on the histology score.

[**0031**] **FIG. 11**: shows the influence of adding a calcium source, a phosphate source, or both a calcium and a phosphate source to the implanted composition at different acidic buffering capacity on the relative mineral mass.

DETAILED DESCRIPTION

[**0032**] The present invention is directed toward an osteogenic product which includes a source of calcium, a source of phosphate and bone growth protein. The osteogenic product is particularly useful in processes of the present invention which include implanting the product in the body for the purpose of inducing bone formation. The present invention provides calcium phosphate ceramic compositions which can be substituted for demineralized bone matrix as a delivery vehicle that not only avoids the risks of transgenic disease/pathogen transmission associated with demineralized bone matrix use, but which also enhances the osteogenic activity of bone growth proteins. Compositions of the present invention have been shown to improve both the quantity (i.e., mass) and quality (by histological score) of bone produced with bone morphogenic protein at reduced doses.

[**0033**] It should be noted that while most contemplated applications of the present invention are concerned with use in humans, the products and processes of the present invention work in non-human animals as well. Induction of bone formation can be determined by a histological evaluation showing the de novo formation of bone with accompanying osteoblasts, osteoclasts, and osteoid matrix. For example, osteoinductive activity of a bone growth factor can be demonstrated by a test using a substrate onto which material to be tested is deposited. For example, osteoinductive activity can be graded or scored as disclosed in U.S. Pat. No. 5,290,763 or as is described below in the Examples.

[0034] A composition of the present invention for bone growth is acidic and includes a substrate, bone growth protein, a source of calcium and a source of phosphate. The substrate provides a structure for the growth of bone and the bone growth protein induces the production of bone. The bone growth protein is highly active in the acidic environment of the composition. The calcium and phosphate sources provide an available supply of these ions for the production of bone.

[0035] The composition of the present invention includes a substrate which provides a structure for the various other components of the composition and also allows for the ingrowth of bone induced by the composition. More particularly, the substrate can be a matrix forming material, such as collagen, fibrin or alginate. A preferred substrate is collagen and a preferred collagen is Type I bovine tendon atelocollagen.

[0036] As used herein, the term bone growth protein refers to a protein or mixture of proteins capable of inducing bone formation when implanted in a body. Suitable bone growth proteins of the present invention can be produced by purification of naturally occurring proteins from bone or by recombinant DNA techniques. As used herein, the term recombinantly produced bone growth protein refers to the production of bone growth protein using recombinant DNA technology.

[0037] A number of naturally occurring proteins from bone or recombinant bone growth proteins have been described in the literature and are suitable. Recombinantly produced bone growth proteins have been produced by several entities. Creative Biomolecules of Hopkinton, Mass., USA produces a bone growth protein referred to as Osteogenic Protein 1 or OP 1. Genetics Institute of Cambridge, Mass., USA produces a series of bone growth proteins referred to as Bone Morphogenic Proteins 1-8 which are described in U.S. Pat. No. 5,106,748. Purified bone growth proteins have been developed by several entities. Collagen Corporation of Palo Alto, Calif., USA developed a purified protein mixture which is believed to have osteogenic activity and which is described in U.S. Pat. Nos. 4,774,228; 4,774,322; 4,810,691; and 4,843,063. Marshall Urist of the University of California developed a purified protein mixture which is believed to be osteogenic and which is described in U.S. Pat. Nos. 4,455,256; 4,619,989; 4,761,471; 4,789,732; and 4,795,804. International Genetic Engineering, Inc. of Santa Monica, California, USA developed a purified protein mixture which is believed to be osteogenic and which is described in U.S. Patent No. 4,804,744. All of the foregoing patents are incorporated herein by reference.

[0038] A preferred bone growth protein of the present invention and process for making the same is described in detail in U.S. Pat. No. 5,290,763, which is incorporated herein by reference. Protein mixtures prepared in accordance with the disclosure of U.S. Pat. No. 5,290,763 are referred to herein as "Bone Protein" or "BP." This bone growth protein is particularly preferred because of its high osteogenic activity and because it is a purified bone growth protein. The Bone Protein of U.S. Pat. No. 5,290,763 exhibits osteoinductive activity at about 3 micrograms when deposited onto a suitable carrier and implanted subcutaneously.

[0039] Yet another embodiment of the preferred bone growth protein of the invention as described in U.S. Pat. No. 5,290,763 includes an osteoinductively active mixture of proteins having, upon hydrolysis, an amino acid composition of from about 20.7 to about 26.1 mole percent acidic amino acids, about 11.3 to about 15.7 mole percent hydroxy amino acids, about 37.6 to about 42.4 mole percent aliphatic amino acids, about 5.8 to about 7.9 mole percent aromatic amino acids and about 13.3 to about 19.9 mole percent basic amino acids. More particularly, the preferred bone growth protein has an amino acid composition of about 20.7 to about 26.1 (preferably about 23.4) mole percent of ASP (+ASN) and GLU(+GLN); about 11.3 to about 15.7 (preferably about 13.5) mole percent SER and THR; about 37.6 to about 42.4 (preferably about 40.0) mole percent ALA, GLY, PRO, VAL, MET, ILE, and LEU; about 5.8 to about 7.9 (preferably about 6.8) mole percent TYR and PHE; and about 13.3 to about 19.9 (preferably about 16.6) mole percent HIS, ARG, and LYS. A further embodiment of the preferred bone growth protein is a protein mixture having the approximate amino acid composition shown in Table 1.

TABLE 1

Amino Acid	Mole Percent
Asp	11.14
Glu	12.25
Ser	9.48
Gly	8.50
His	2.28
Arg	7.19
Thr	4.03
Ala	8.05
Pro	7.16
Tyr	3.63
Val	3.79
Met	1.73
Ile	2.75
Leu	8.00
Phe	3.21
Lys	7.11

[0040] In a further embodiment, the bone growth protein of the present invention is a "TGFβsuperfamily protein" which can be any protein of the art-recognized superfamily of extracellular signal transduction proteins that are structurally related to TGFβ1-5. Preferably, a TGFβ superfamily protein suitable for use in the present invention is selected from the following proteins: TGFβ1, TGFβ2, TGFβ3, bone morphogenic protein (BMP)-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-9, cartilage-derived morphogenic protein (CDMP)-1, CDMP-2, and/or CDMP-3.

[0041] The amount or dose of bone growth protein used depends on the activity of the bone growth protein and the particular application. In the case of the bone growth protein identified in U.S. Pat. No. 5,290,763, the bone growth protein is used in amounts between about 10 micrograms/gram substrate and about 10,000 micrograms/g substrate and more preferablybetween about 100 micrograms/g substrate and about 350 micrograms/g substrate.

[0042] It has been determined by the present inventors that solution pH plays a strong role in the osteogenic performance of bone growth proteins, with acidic environments providing dramatically superior results. As such, a further aspect of the composition of the present invention is the

acidic buffering potential in physiological solutions. More particularly, when the composition of the present invention is put into a solution, such as a bodily fluid at physiological pH (e.g. in an in vivo application) or another weakly basic solution, the composition acts to buffer the solution at an acidic pH (i.e., the pH of the composition is less than 7). Additionally, if the composition is implanted into a mammal, the composition can buffer the surrounding microenvironment to an acidic pH. More particularly, the present compositions can buffer such solutions in the immediate environment to a pH between about 4 and about 7, more preferably between about 5 and about 6.8, and most preferably between about 5.5 and about 6.7. For example, such slightly basic solutions can include in vivo environments, having a pH of approximately 7.4 or a normal saline solution having a similar pH. Control of the pH of the compositions can be achieved by those skilled in the art using routine techniques. For example, the use of buffering agents to maintain a desired pH range is well-known. Obviously, since compositions of the present invention are intended for in vivo application, such buffering agents need to be biocompatible. Particularly preferred buffers are discussed in more detail below.

[**0043**] The composition of the present invention also includes sources of calcium and phosphate. These sources are used to locally enhance the soluble concentration of dissolved calcium [Ca^{2+}] and phosphate [PO_4^-] around the site of implantation of the composition. Natural bone acts as a reservoir to maintain constant serum concentrations of these components. It has been theorized that the rate of bone formation is limited by the diffusion of these critical ions to the site of bone induction. In brief, bone mineralization exhausts the local serum concentration of calcium and phosphate, after which bone mineralization is limited by the rates of both osteoclast resorption of local bone (to provide soluble calcium and phosphate ions) and diffusion of these ions to the site of bone induction. By specifically enhancing the local concentration of these critical components, such as by the use of sparingly soluble calcium phosphate additives, the amount (mass) and quality of induced bone formation can be enhanced.

[**0044**] Suitable sources of calcium for the composition of the present invention include essentially any calcium salt, including calcium phosphate or calcium citrate. Particularly preferred sources of calcium include calcium phosphate salts, and more particularly preferred sources of calcium include acidic calcium phosphate salts. Preferred acidic calcium phosphate salts include calcium monophosphate, calcium hydrogen phosphate, and calcium pyrophosphate. Typically, the calcium source is present in the composition in an amount of between about 1% by weight and about 85% by weight.

[**0045**] Suitable sources of phosphate for the composition of the present invention include essentially any phosphate salt, including calcium phosphate or sodium phosphate. Particularly preferred sources of phosphate include calcium phosphate salts, and more particularly preferred sources of phosphate include acidic calcium phosphate salts. Preferred acidic calcium phosphate salts include calcium monophosphate, calcium hydrogen phosphate and calcium pyrophosphate. Typically, the phosphate source is present in the composition in an amount of between about 1% and about 75%.

[**0046**] As noted above, preferred sources of calcium and of phosphate include acidic calcium phosphate salts. Calcium phosphates are represented by the general chemical formula of $xCaO \prec P_2O_5$. The sparingly soluble calcium phosphate salts act as solution buffers. As the salts increase in calcia concentration (CaO), the pH increases from approximately 2 (x=1) to 11 (x=4). It is believed that the alkaline buffering nature of hydroxyapatite (x=3.33) actually reduces the performance of bone growth proteins. It has been found that acidic calcium phosphate salts (i.e., calcium monophosphate ($Ca(H_2PO_4)_2$), calcium hydrogen phosphate ($CaHPO_4 \cdot 2H_2O$) and calcium pyrophosphate ($2CaO \cdot P_2O_5$)) stimulate the osteogenic performance of bone growth protein. In comparison to collagen composites containing devitalized bone matrix additives, collagen dispersions containing calcium hydrogen phosphate ($CaHPO_4 \cdot 2H_2O$) have resulted in superior bone quality.

[**0047**] Thus, in preferred embodiments, the composition of the present invention utilizes calcium phosphate salts and/or calcium containing pH buffer salts to (1) control local pH (to enhance/control bone growth factor release mechanisms), (2) locally enhance soluble calcium concentration (which increases bone production), and (3) locally enhance soluble phosphate (which also increases bone production). In comparison to collagen composites containing devitalized bone matrix additives, collagen dispersions containing acidic calcium phosphates have been developed that stimulate the formation of larger explants containing bone of superior quality. As noted above, control of each of the foregoing three factors independently can be used to enhance the activity of bone growth proteins. Accordingly, acidic mineral salts other than calcium phosphate salts can be used to control pH, thereby increasing the bone morphogenic activity of bone growth proteins without providing additional calcium or phosphate. Additionally, other buffering agents (e.g. a sulfate-based buffer) or acidifying agents (e.g. lactic acid) can be used to control the local pH surrounding the composition in the absence of a calcium source, in the absence of a phosphate source, or in the absence of a calcium phosphate source. Similarly, the use of specific calcium salts (e.g. calcium citrate) which do not incorporate phosphorus can be used without regard to control of local pH or phosphate concentrations. Likewise, the use of non-calcium phosphate salts (i.e., sodium phosphate salts) can be used to enhance local concentrations of phosphate ions to enhance bone morphogenic activity without specifically controlling local pH or calcium concentrations. Each of these three factors (the additions of a calcium source, the addition of a phosphate source, and the control of the local pH) leads to increased bone production or growth independently of one another as shown in **FIGS. 10 and 11**. The bone growth and production enhanced by these three factors may be increased in quantity (as evidenced by increased relative mineral mass) or quality (as evidenced by increased relative histology score) or may be increased in both quantity and quality. Furthermore, the effects on bone growth enhanced by these three factors are separately additive. Thus, the combination of any two of the three factors in the final composition will increase the production of bone above the bone growth seen with any one of the factors independently.

[**0048**] The composition of the present invention can be in a variety of different forms. In a preferred embodiment, a collagen sponge is provided which contains bone growth

proteins as well as calcium phosphate salts for controlling pH and providing calcium and phosphate to the local environment. An example of how to prepare such a sponge is provided below.

[0049] Another embodiment of the present invention is a novel process to produce collagen sponges for implantation which incorporate the synthetic replacement materials generally described above. The products are prepared by producing a dispersion of collagen fibrils that contains either solubilized calcium salts or solubilized phosphate salts. Suitable collagen can include type I collagen, type II collagen, type III collagen, or type IV collagen. In one embodiment, the collagen is from bovine tendon. The collagen dispersion is typically between about 0.5% by weight and about 20% by weight collagen, more preferably between about 1% by weight and about 10% by weight collagen, and most preferably between about 3% by weight and about 5% by weight collagen.

[0050] If the dispersion was made with a calcium salt, a phosphate salt is then added to the dispersion to heterogeneously precipitate a calcium phosphate salt directly onto the surface of the collagen fibrils. If the dispersion was made with a phosphate salt, a calcium salt is then added to the dispersion to heterogeneously precipitate a calcium phosphate salt directly onto the surface of the collagen fibrils. The interfacial adherence of the precipitate improves the mechanical rigidity and wetability of the composite sponges. The application of dehydrothermal collagen cross-linking techniques (e.g., 110° C., 24-72hrs, vacuum) are well known in the art. Such cross-linking techniques result in the formation of water stable, collagen sponges of superior physical properties. Such sponges can then be loaded with bone growth protein and used for induction of bone growth in vivo. In a preferred embodiment, the products are prepared by producing a 4% (by weight) collagen dispersion that contains solubilized calcium dichloride dihydrate ($CaCl_2 \cdot 2H_2O$). A solution of disodium phosphate (Na_2HPO_4) is added to the heterogeneously precipitate calcium hydrogen phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$) directly onto the surface of collagen fibrils.

[0051] Another process of the present invention includes implanting a composition as broadly described above into a body for induction of bone growth. As noted above, most uses of the present invention are concerned with human applications. The process, however, is suitable for a wide variety of animals, particularly including other mammals. As used herein, the term implanting refers to placing the composition of the present invention in any bone defect or other area in which it is desired to have bone grow. By implanting composition, bone formation is induced by the bone growth protein. Over time, preferred calcium and phosphate materials are resorbed allowing for uniform bone formation throughout a defect area.

[0052] Compositions of the present invention can be used in a variety of applications whenever there is a need to generate bone. Such applications include induction of bone formation for hip replacement operations, knee replacement operations, spinal fusion procedures, repair of periodontal defects, treatment of osteoporosis, repair of bone tumor defects and repair of bone fractures.

[0053] In the case of hip replacement operations, the ball and socket joint of a hip is replaced when a person's hip is not functioning properly. The ball portion of a joint is replaced by surgical removal of the ball portion from the terminus of the femur. The artificial ball portion has a functional ball end with the opposite end being a spike which is inserted into the proximal end of the femur from which the natural ball portion was removed. The spike can have a porous surface so that bone growth around the spike can anchor the spike in the femur. The product of the present invention, in particulate form, is layered or packed between the spike and the cavity in the femur in which spike is to be inserted. The socket portion of a joint is replaced by inserting an artificial socket into the natural socket. The artificial socket is sized to fit with the artificial ball. On the surface of the artificial socket which contacts the natural socket, the artificial socket can have a porous surface. The product of the present invention, in particulate form, is placed in the natural socket cavity so that upon placement of the artificial socket, the product is between the natural and artificial socket. In this manner, as bone is formed, the artificial socket is anchored in the natural socket.

[0054] Products of the present invention are also suitable for use in knee replacement operations. Knee prostheses have a femoral and a tibial component which are inserted into the distal end of the femur and the surgically prepared end of the tibia, respectively. The product of the present invention, in particulate form, is layered or packed between the femoral and/or tibial components of the prosthesis and the respective portions of the femur and tibia. In this manner, as bone formation is induced between the prosthesis and the bones, the prosthesis becomes anchored.

[0055] Products of the present invention are also suitable for use in spinal fusion operations in which it is desired to substantially immobilize two vertebrae with respect to each other. The product can be applied, for example, between adjacent spinous and transverse processes so that upon bone formation throughout the composite material, two adjacent vertebrae are joined by fusion between the respective spinous processes and transverse processes.

[0056] In the case of periodontal defects, the product of the present invention is conformed to the defect shape. As bone growth is induced, bone fills in the defect.

[0057] In the treatment of osteoporosis, the present product is injected in existing bone to offset the effects of osteoporosis in which bone density is lost. For example, if it is determined that bone density is low in a localized area, such an injection can be made in that area.

[0058] The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

Example 1

[0059] This example illustrates the production and use of synthetic bone growth protein containing devices that provide equivalent or superior osteogenic performance without the addition of demineralized bone matrix additives as biologic supplements.

[0060] This example shows the influence of the carrier vehicle on the in vitro and in vivo osteoinductivity attributable to osteogenic growth factors. An accepted protocol to

assess the osteogenic activity of composite materials is through implantation of samples in rats. The advantages of the rat model for product evaluation include its moderate cost and an accelerated rate of bone induction. Visible evidence of mineralization appears in the implant within several days (~10), with typical experiments lasting between 14 and 21 days. Osteogenic activity is commonly evaluated using four standard test protocols: histological tissue analysis, mineral concentration via x-ray and ash weight evaluation and bone cell activity via alkaline phosphatase analysis.

[**0061**] This example specifically compared the osteogenic differences between implant samples containing Bone Protein (BP), collagen (bovine tendon type 1) and a powder of either devitalized bone matrix (DVBM) or the synthetic calcium phosphate ceramic (Ostite) [Millennium Biologix, Kingston, Canada]. Ostite is a synthetically derived material containing variable concentrations of calcium hydroxyapatite and silica stabilized tricalcium phosphate. Similarly to alternative calcium phosphate sources, Ostite supports the required interfacial activity of osteoblasts for bone regeneration. The unique advantage of Ostite is that it has been shown to degrade only by osteoclastic resorption. Sample disks were prepared with variable composition: a) collagen (100wt. %)/BP and b) collagen/particle (50/50wt. %)/BP. The sample disks were prepared using two distinct processing techniques. In the first technique, the components were mixed in phosphate buffered saline (PBS) at a collagen ratio of 4 wt. %. The mixtures were molded into disks (h~3 mm, d~8 mm) and freeze dried. In the second technique, the components were mixed with dilute acetic acid (1 vol. %) to form a gel with a collagen ratio of 4 wt. %. The gels were molded into disks and freeze-dried. All disks were loaded with BP and freeze dried according to standard protocols.

[**0062**] The testing protocol involved sample implantation in subcutaneous (to assess endochondral bone formation) and calvaria sites (to assess membranous bone formation). The osteoinductive responses were evaluated after 4 weeks implantation using accepted protocols for explant mass, ash weight, x-ray mineral density and histology.

[**0063**] Clinically, the application of bone morphogenic proteins (BMPs) and other osteogenic growth factors are desired to assist in the surgical reconstruction of skeletal defects. BMPs are advantageous because they induce bone formation by targeting and activating undifferentiated perivascular connective tissue cells. In contrast, growth factors (i.e., mitogens) target and accelerate the osteogenic activity of previously differentiated cells. Numerous advances have improved the activity of osteogenic factors, however, their clinical application has been limited by the requirement for a superior delivery vehicle.

[**0064**] PROCEDURES:

[**0065**] Collagen Implants:

[**0066**] Collagen sponge disks were prepared according to standard procedures as follows; Mix 12.0 g of 1 vol. % glacial acidic acid and 500 mg of accepted Bovine tendon Type 1 Collagen in an inert screw cap container. Mix with a spatula as the gel begins to form, minimizing the number of trapped air bubbles. Stop mixing when the gel becomes thick. Tap gel container on bench-top to remove trapped air bubbles and cap tightly. Allow mixture to sit for at least 1 hour at room temperature.

[**0067**] To make disks from the collagen dispersion, place a Delrin disk mold sheet on a glass plate and press the dispersion into the holes. Remove excess dispersion with a knife or spatula. Place the molding sheet and glass plate in a freezer at –80C for approximately 1 hour. Remove from the freezer and allow warming for approximately 1 minute. Remove the glass plate and place the Delrin plate into a freeze-drying flask. Freeze dry for a minimum of 12 hours. After drying, remove the samples from the plate, trim the edges and weigh each disk. Each disk must weigh between 6.5 to 7.3 mg to be acceptable for use.

[**0068**] Collagen/Powder Implants:

[**0069**] In an inert screw cap container, mix 600 mg of accepted Bovine tendon Type 1 collagen with 600 mg of either Ostite powder (NP) or devitalized rate bone matrix powder (NP). Add 14.4 g of acetic acid (1 vol. %) to prepare gel dispersions containing 4 wt. % collagen. Stir with a spatula to homogenize the mixtures and to adequately wet the components. Vibrate the mixtures on a high intensity orbital shaker to remove trapped air bubbles. Allow mixtures to sit for at least 1 hour at room temperature.

[**0070**] To make disks from the collagen dispersions, place a Delrin disk mold sheet on a glass plate and press the mixtures into the holes. Remove excess mixture with a knife or spatula. Place the molding sheet and glass plate in a freezer at –80° C. for approximately 1 hour. Remove from the freezer and allow warming for approximately 1 minute. Remove the glass plate and place the Delrin plate into a freeze-drying flask. Freeze dry for a minimum of 12 hours. After drying, remove the samples from the plate, trim the edges and weigh each disk. The disks must weigh between 13.0-14.6 mg to be acceptable for use.

[**0071**] BP Loading:

[**0072**] Dilute a volume of BP (produced as described in U.S. Pat. No. 5,290,763) with a volume of 10 mM HCI to prepare solutions of 10 mg BP/100 ml (15 ml) and 35 mg BP/100 ml (4.0 ml). In the Delrin loading plate, pipet 50 ml of a solution on the top and bottom half of a collagen sponge (n=240 (10 mg), n=48 (35 mg)). Allow disks to stand in a chamber containing a moist paper towel (to prevent drying and sponge shrinkage) at ambient temperatures for 40-60 minutes. Cover the disk holding plate with Saran Wrap and place in a –80° C. freezer for 40-60 min. Unwrap and carefully place in a freeze dryer flask. Freeze dry for a minimum of 12 hours then remove. The implant samples will respectively contain total BP masses of 10 and 35 g.

[**0073**] Surgical controls were used to determine the osteogenic response in the calvaria implants due to irritation of the periosteum. A solution of 10 mM HCI was prepared and sterilized by filtration through a 0.2 mm sterile syringe filter. The solution was applied to the collagen disks in an identical manner as the BP loaded samples and served as negative controls.

[**0074**] Sample Disk Implantation

[**0075**] The weight of each Long-Evans rat was recorded. Acceptable rats for bioassays weigh between 100 and 130g. The animals was anesthetized with 400 ml of pentabarbital dosing solution injected i.p..

[**0076**] Subcutaneous sample implantation is made as follows: make small (6 mm) incisions in the skin of the ventral

or dorsal thorax. Ventral incisions were made at the base of the rib cage. A template, to be aligned with the base of the rib cage, was provided to identify constant dorsal implant locations. After incision, a pocket beneath the skin and above the incision were prepared by blunt dissection. Place the loaded collagen sponges in the pocket, approximately 5 mm above the incision. Repeat additional incisions and implant insertions then close the incisions with Tedvek II 5-0 (or equivalent) sutures.

[**0077**] House the animals in compliance with the guidelines described in QC-008. Check animals for lesions 3-5 days post implantation. If lesions are detected or if animal death occurs before sacrifice, document results.

[**0078**] Implantation Protocol and Analysis

[**0079**] The testing protocol involved subcutaneous implantation of collagen sponges (to assess endochondral bone formation) containing 10 g BP. The samples were placed in four subcutaneous implantation sites: the upper quadrants of a rat's abdomen and dorsal thorax [FIG. 1]. In addition, the testing protocol involved calvaria implantation of collagen sponges (to assess membranous bone formation) containing either 0 mg or 35 mg BP. Samples of variable composition and concentration can be produced.

[**0080**] The osteogenic activity of the implant is evaluated using accepted protocols for explant mass, ash weight, x-ray mineral density and histology. A total of 20 rats/composition were utilized. This population provided location-specific testing numbers of n=10/test for the subcutaneous assays. Normalizing the samples according to location-specific values provides a total subcutaneous sample population of n=40/test.

[**0081**] Three weeks post implantation, the animals (n=20) were sacrificed through CO_2 asphyxiation. The weight of the host rat and each implant is immediately measured post-surgical excision. The explants are imaged with x-ray radiation to determine mineral density as a function of composition and implant location. 40% of the subcutaneous samples were analyzed using accepted protocols for ash weight.

[**0082**] The remaining subcutaneous explants were analyzed for differences in tissue quality using accepted histology protocols. The averaged results and their standard deviations were analyzed for statistical significance using ANOVA comparisons. The results are shown in FIGS. **2-5**.

Example 2

[**0083**] This example illustrates the independent effects of a calcium source and a phosphate source in the present invention.

[**0084**] In vivo rat implantation assays were conducted to determine the effects of local supplementation of calcium, phosphate, and of both calcium and phosphate in the implanted compositions of the present invention. The implants containing a calcium source, a phosphate source or a source of both calcium and phosphate were tested and evaluated in terms of relative histology score and relative mineral mass gain. The results of these assays are shown in **FIGS. 10 and 11**.

[**0085**] While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.

What is claimed is:

1. A bone growth composition, comprising:

(a) a substrate;

(b) bone growth protein;

(c) a source of calcium; and,

(d) a source of phosphate, wherein said composition has an acidic buffering potential in physiological solution.

2. A bone growth composition, comprising:

(a) a substrate;

(b) bone growth protein; and,

(c) a source of calcium,

wherein said composition has an acidic buffering potential in physiological solution.

3. A bone growth composition, comprising:

(a) a substrate;

(b) bone growth protein; and, (c) a source of phosphate,

wherein said composition has an acidic buffering potential in physiological solution.

4. A bone growth composition as claimed in claim 1, wherein the source of calcium is an acidic calcium phosphate salt.

5. A bone growth composition as claimed in claim 4, wherein the source of calcium is selected from the group consisting of calcium monophosphate, calcium hydrogen phosphate, and calcium pyrophosphate.

6. A bone growth composition as claimed in claim 1, wherein the source of phosphate is a sodium phosphate salt.

7. A bone growth composition as claimed in claim 1, wherein the substrate is selected from the group consisting of collagen, fibrin, alginate and mixtures thereof.

8. A bone growth composition as claimed in claim 1, wherein the bone growth protein is selected from the group consisting of purified bone growth factors, recombinantly produced bone growth factors and mixtures thereof.

9. A bone growth composition as claimed in claim 8 wherein the bone growth protein comprises a transforming growth factor β(TGF-β) superfamily protein.

10. A bone growth composition as claimed in claim 8 wherein the bone growth protein comprises Bone Protein.

11. A process for producing an implantable bone growth composition, comprising:

(a) producing a dispersion of collagen fibrils containing a solubilized sodium phosphate salt; and

(b) adding a calcium chloride salt to the dispersion of collagen fibrils to precipitate a calcium phosphate salt onto the surface of said collagen fibrils to produce an implantable bone growth composition.

12. The process of claim 11, wherein said solubilized sodium phosphate salt is calcium hydrogen phosphate dihydrate and wherein said calcium phosphate salt is calcium dichloride dihydrate.

13. A process for producing an implantable bone growth composition, comprising:

(a) producing a dispersion of collagen fibrils containing a solubilized calcium chloride salt; and,

(b) adding a sodium phosphate salt to the dispersion of collagen fibrils to precipitate a calcium phosphate salt onto the surface of said collagen fibrils to produce an implantable bone growth composition.

14. The process of claim 13, wherein said solubilized sodium phosphate salt is calcium hydrogen phosphate dihydrate and wherein said calcium phosphate salt is calcium dichloride dihydrate.

15. A process for the induction of bone formation in a mammal, comprising implanting a bone growth composition in said mammal, wherein said composition comprises,

(a) a substrate;

(b) bone growth protein;

(c) a source of calcium; and,

(d) a source of phosphate,

wherein said composition has an acidic buffering potential in physiological solution.

16. A process as claimed in claim 15, wherein said source of calcium is an acidic calcium phosphate salt.

17. A process as claimed in claim 16, wherein said acidic calcium phosphate salt is selected from the group consisting of calcium monophosphate, calcium hydrogen phosphate, and calcium pyrophosphate.

18. A process as claimed in claim 15, wherein said source of phosphate is a sodium phosphate salt.

19. A process as claimed in claim 15, wherein said substrate is selected from the group consisting of collagen, fibrin, alginate and mixtures thereof.

20. A bone growth composition as claimed in claim 15, wherein the bone growth protein is selected from the group consisting of purified bone growth factors, recombinantly produced bone growth factors and mixtures thereof.

21. A bone growth composition as claimed in claim 20 wherein the bone growth protein comprises a transforming growth factor β(TGF-β) superfamily protein.

22. A bone growth composition as claimed in claim 20 wherein the bone growth protein comprises Bone Protein.

23. A process as claimed in claim 15, wherein said process is a process selected from the group consisting of hip replacement operation, knee replacement operation, spinal fusion, repair of periodontal defects, treatment of osteoporosis, repair of bone defects and repair of bone fractures.

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