

The Significance of Zeta Potential in Osteogenesis

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Introduction

A direct link between an electric effect and a physiological effect was first established by Luigi Galvani in 1791 in the famous 'frogs legs' experiment. The first reported successful treatment of bone non-unions by electric fields/currents was by Hartshorne¹ and Lente². Eriksson³ reported on the relationship of surface energies and charge effects on the bone induction principle. Krukowski in a series of papers^{4,5,6} demonstrated a significant *in-vivo* response of both hard and soft-tissue to charged resin beads and Eriksson in 19767, using the demineralised bone samples prepared and used by Urist⁸ in his seminal study, demonstrated that the materials having the highest osteoinductive potential had the greatest negative surface electric charge. Under physiological conditions, calcium phosphate bone graft materials would typically show a positive zeta potential.

In an aqueous environment all cells normally exhibit a negative surface charge. Proteins are colloidal particles having charged surfaces. Implant materials can also exhibit surface electric charge in an aqueous environment. It is natural to assume that the charge on an implanted material in some way or other has an influence on the behaviour of the body's cells towards that material. It is an objective of the present study to assess the response of osteoblasts to calcium phosphate bone graft materials having negative and positive zeta potential.

Materials and Methods

Tricalcium phosphate (TCP) and hydroxyapatite (HA) granules were prepared having differences in surface chemistry which resulted in materials with both positive and negative zeta potential values (estimated at + and - 30mv). Human osteoblasts at 50,000 cells/ml were seeded onto the various materials in standard culture wells and the response was visually assessed after time periods of 1, 2 and 3 days in culture at 37°C. Gene expression of osteogenic markers, alkaline phosphatase, osteocalcin, osteopontin, CBFA1 and collagen type 1 was determined by RT-PCR after the 3-day culture period.

Results and Discussion

Significant differences in the behaviour of osteoblasts to positive and negatively charged surfaces were immediately observed (1 day culture). Both HA and TCP with negative zeta potential showed florid osteoblast activity. They were more amenable to osteoblast attachment and proliferation than positively charged samples. Figures 1 and 2 taken at 3 days culture (Stain, Toluidine Blue) show osteoblast activity surrounding HA particles of negative and positive charge respectively. Figure 1a is a higher magnification view of an osteoblast seen bridging the HA particle and the culture well. Table 1 shows the gene expression data for the positive and negatively charged TCP. The negatively charged material shows enhanced up-regulation of all five osteogenic markers (relative to a standard marker gene) compared to positively charged material. Since osteoblasts are negatively charged, and since like-charges repel, then it must be assumed that serum proteins initially adsorbed onto the calcium phosphate are responsible for subsequent cellular activity. The relative iso-electric points of the biomaterial, the cell and the proteins could be an indicator of morphogenetic activity and a significant factor in the bone-healing cascade.

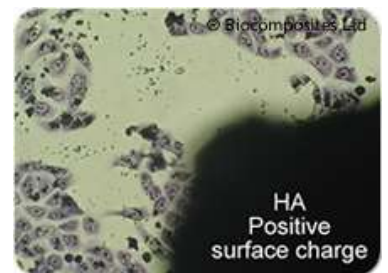


Figure 1
 At 3 days culture (Stain, Toluidine Blue) show osteoblast activity surrounding HA particles of negative charge

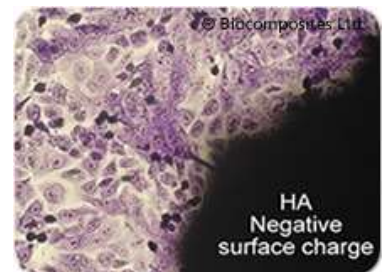


Figure 1a
 A higher magnification view of an osteoblast seen bridging the HA particle and the culture well

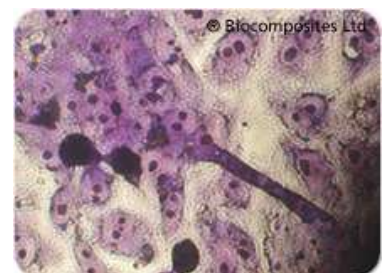


Figure 2
 At 3 days culture (Stain, Toluidine Blue) show osteoblast activity surrounding HA particles of positive charge

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Conclusions

The zeta potential of calcium phosphate bone void fillers has been shown to have biological significance *in-vitro*. The use of alloplastic materials having negatively charged surfaces for the repair and augmentation of bone warrants further investigation.

Future work

Studies are continuing in order to establish statistical significance to the results seen so far and to extend the work *in-vivo* to see if the enhanced osteoblast response to negatively charged surfaces translates to enhanced osteogenic activity.

BONE PROTEIN	β TCP POSITIVE CHARGE	β TCP NEGATIVE CHARGE
Alkaline Phosphate	++	++
Osteocalcin	○	++
Osteopontin	++	+++
CBFA1	--	++
Collogen Type 1	-	+

Table 1
The use of "+" and "-" indicates the relative quantities of protein upregulated or down-regulated.

References

1. Hartshorne E, Am J Med. 1841; 1: 121-156.
2. Lente RW, NY State J Med. 1850; 5: 317-319.
3. Eriksson C, J Biomed Mats Res. 1985; 19: 833-849.
4. Krukowski M, J Oral Max Surg. 1990; 48: 468-475.
5. Krukowski M, Clin Orthop Rel Res. 1994; 298: 266-271.
6. Krukowski M, Plast Reconstr Surg. 1992; 89: 891-897.
7. Eriksson C, Clin Orthop Rel Res. 1976; 121: 295-302.
8. Urist M, Science. 1965; 150(698): 893-899

