The Effects Of Amino Acid Binders On Proliferation And Viability Of Fibroblast Cells

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Introduction: A prosthetic joint is not as strong or as durable as a healthy joint, and the longevity varies from patient to patient. Several physiological, surgical or biological factors determine the degree of osseointegration and longevity of the implant. In most cases bone is separated from the implant material by a very thin biological layer. However, in some genetic and physiological states the fibrous tissue may intervene between the implant and the body and compromise the success of the implant. Bioceramic coatings may be able to reduce the formation of fibrous tissues. Among the ceramics that have been studied, tricalcium phosphate (TCP) tends to exhibit these characteristics. The properties of TCP allow for potential use as scaffolds for cells in the field of tissue engineering. Changing the formation of traditional ceramics to include amino acids with different pKa side chains will significantly affect the cellular microenvironment and ultimately the interactions at the cell implant surface. The specific aims of this investigation were: (i) to formulate ceramic devices with different amino acid binders and characterize the material for density and amino acid content, (ii) to assess the fibroblast density around TCPL-amino acid bioceramics, and (iii) to evaluate the cellular response associated toward the amino-acid binders.

Material and Methods:

Fabrication of Ceramics: Microcrystals of calcium phosphate were prepared by following standard laboratory protocols (Benghuzzi et al (1990). The sintered material (300mg of TCP) was added to a total of 50 mg of an amino acid binder except for the TCP control, which contained 350 mg of TCP with no amino acid binder. The following amino acids were used as binders: lysine (lys), aspartic acid (asp), cystine (cys), histidine (his), and serine (ser). The combination of TCP and amino acid binders were then cold-pressed into cylindrical form using a 3/8 inch die set at a compression of 2500 kg. The polar, hydrophilic amino acids chosen for this experiment had pKa values ranging between 3.90 and 13.

Cell Line: MRC-5 fibroblasts were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

Experimental Design: MRC-5 cells (50,000 cells/well) were grown in wells containing amino-acid ceramics for 24, 48, and 72 hours. At the end of the each phase the cells were counted and evaluated for damage, total protein, and viability.

Cell Counts: The ceramic devices were removed, and the cells collected. The cells were washed then resuspended in 400ul of phosphate buffered saline (PBS, pH 7.2). Cell counts were determined using standard hemacytometer techniques.

Protein: Pierce's Bicinchoninic Acid (BCA) microassay was used for cellular protein evaluations. Ten microliters of cells, or protein standard solution was combined with BCA and incubated at 27°C for 1-hour. Absorbency was recorded at 560 nm, and cellular concentration determined based upon the slope and intercept of the standard curve.

Maliondialdehyde (MDA): Cellular damage by determined by an end-point determination assay measuring thiobarbituric reactive substances (Maliondialdehyde bis diethyl acetyl).

Morphological Evaluations: MRC-5 Fibroblasts were stained using standard Hematoxylin and Eosin staining procedures. Light microscopy was used to evaluate morphological changes.

Statistics and Graphics: Descriptive statistics and analysis of variance were performed using Sigma Stat Software.

Results and Discussion: Cell number: Amino acids immobilized on surfaces of various types of implants have been shown to markedly affect the rate of attachment as well as cell growth (Ito et al (1997); Higuchi et al (2000); and Poiani et al (1997)). Fibroblast cells at 24 hours showed significant increases in cell number when compared to the control. This data coincides with reports in the literature for various amino acids (Ito et al (1997, Higuchi et al (2000), and Poiani et al (1997)). With increases in culture time, these differences were not as striking. Lysine used as a binder showed the greatest numbers at 48 and 72 hours compared with other groups (Figure 1). This trend was expected since lysine has a charged R group similar to arginine and aspartic acid which has been shown to promote tissue adherence. Interestingly, the higher the R group pK_R the greater the attachment (Asp, Lys and Ser) This information also confirms previous studies using lysine in adhesion of monocytes and HeLa (Benghuzzi and Tucci (1995); Benghuzzi (1995)).

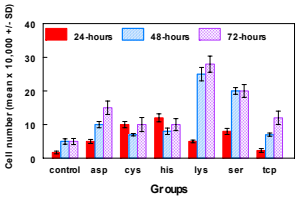

Figure 1. Cell number is shown for 24, 48 and 72 hours. The results are an average of 3 separate experiments (n=6 per

Cellular protein levels: Cells exposed cys-ceramics had markedly increased levels of protein for the duration of the study. Cells adhered to lysine and serine bioceramics were moderately increased for the first 48 hours. Serine and lysine are amino acids with very basic R-groups and very similar pKa values for both the carboxylic and amino groups. These characteristics may be important factors for cellular interaction.

Cellular MDA: MDA is a marker for cell membrane damage that measures lipid peroxidation ($LOO^{\cdot}$). $LOO^{\cdot}$ can perturb membrane structure/function and can be deleterious to cells [Girotti, 1998]. The data obtained indicated TCP alone did not cause drastic increases in $LOO^{\cdot}$ moieties with time (Figure 2). Increases in MDA levels were observed in cells attached to cys bioceramics at 24 hours, but the increase was not evident at 48 and 72 hours.

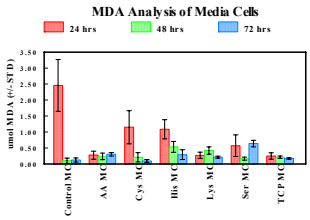

Figure 2. MDA levels are shown for 24, 48 and 72 hours. The results are an average of 3 separate experiments (n=6 per group)

Conclusions: Amino acids such as arginine, glycine and aspartic acid have been shown to be the recognition sites for attachment by fibroblasts. The amino acid binders did not alter the membrane properties beyond the level of cellular recovery as evidenced by similar MDA levels at 48-72 hours. The information provides evidence for the use of natural compounds as additions for fabrication of biomedical implants

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