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Research Article

A Study to Investigate the Osteogenic Potential of Peptide AC-100

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Abstract

Introduction: Titanium alloy is a commonly used implant material in orthopaedics but improving its interface with bone is a key goal in its application to new tissue engineering approaches. In this project we investigated the osteogenic potential of AC-100 (an active motif from the Matrix Extracellular Phosphoglycoprotein) and in doing so further studies could be carried out on titanium surfaces.

Methods: AC-100 peptide was attached to glass cover slips to influence rat calvarial osteoblast adhesion and compared it to adsorbed fibronectin (FN) and plain glass (control surface).

Results: The results were analysed with ANOVA. Glass surfaces coated with AC-100 and FN showed osteoblast adhesion and spreading significantly greater than the control surface ($p < 0.05$).

Conclusion: We demonstrated that AC-100 when compared to Fibronectin provides a suitable environment for osteoblast adhesion on glass surface, which may translate to offering a suitable environment for osteoblast adhesion on titanium.

Keywords: *Fibronectin; C-100- an active motif from the Matrix Extracellular Phosphoglycoprotein; Ti: Titanium, FN: Fibronectin; Level of Evidence -II*

Abbreviations

PA: Phosphonic acid; FN: Fibronectin; HA: Hydroxyapatite; Robs: Rat Calvarial Osteoblasts; PBS: Phosphate buffer solution; EDC: 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride; Sulpho-NHS: N-hydroxysulfosuccinimide; MES: 2-(N-morpholino) ethanesulfonic acid; RGD: Arginine-Glycine-Aspartate Peptide; ECM: Extracellular Matrix; DMEM: Dulbecco's Modified Eagle's Medium; BSA: Bovine Serum Albumin; DAPI: 4',6-diamidino-2-phenylindole

Introduction

The published research articles studied the surface topography of the titanium and proved that roughness of the surface affects the phenotype of osteoblasts by promoting differentiation, whilst down-regulating cell proliferation [1-3]. Regarding surface chemistry of the metal more research is necessary. Cementless implants are often coated with Hyrdroxyapatite [HA], to promote osteoblast adhesion; HA is commonly coated onto the implant surface by plasma deposition, which can be susceptible to degradation in physiological conditions [4,5].

This study looks to investigate the osteogenic potential of peptide AC-100 (an active motif from the Matrix Extracellular Phosphoglycoprotein) and in doing so may be a suitable alternative or maybe used in conjunction to HA in future applications.The aim of the study was to immobilise the peptide AC-100 onto glass cover slips and investigate osteoblast adhesion onto the surface and compare it to Fibronectin (a positive control) and plain glass control surfaces.

Materials and Methods

The experiments involving live cells were carried out with rat calvarial osteoblasts (RObs) using a class II tissue culture hood under aseptic conditions. Ethanol was used as steriliser to all the surfaces before and after experiments and with the apparatus.

The culture media utilised was Dulbecco's Modified Eagle Medium (DMEM. Phosphate buffer solution (PBS), without calcium and magnesium ions were utilised along with 0.05% Trypsin with 0.53 mM EDTA ,whch were used to detach cells adhered to culture plates and 1mg/ml of trypsin inhibitor from soya beans were also utilised. Incubators were set at 37 oC with 5% carbon dioxide in a humidified atmosphere. Cells were stained prior to microscopy using 0.1% v/ml phalloidin stain (prepared by mixing 2.5µl of phalloidin in 2.5ml of PBS tween) and 1ml of Vectashield with DAPI stain.

The peptide AC-10 with an amino acid sequence ERGDNDISPFSGDGQ, 0.1% bovine fibronectin (FN) were utilised. A positive control surface, modified with FN and a negative control of untreated glass were created and modified surface AC-100cover slips were created.

RObs were retrieved from an incubator and the growth media was removed, using a pipette and placed in a waste beaker. The amount of cells required for the experiment (50,000 cells per surface) were used and the cells that remained were transferred into another culture flask with DMEM media and returned to the incubator.

The sterile AC-100 coated cover slips were added with 0.5ml BSA. BSA was also added to the RObs that were passaged and 0.5ml of the solution containing the RObs were distributed into each well. The wells were incubated for 24 hours.

The plates were retrieved from the incubator under sterile conditions. The surfaces were washed three times with 0.1% PBS tween (PBS + detergent). 300µl of phalloidin stain was added to each well for 20-30mins. The surfaces were washed three times with PBS tween; the last wash was left on. Using tweezers, the cover slips were washed in distilled water and dried and then placed face down on microscope slides which had a drop of DAPI stain on them.

Results

The surfaces were examined under an epi-fluorescence microscope and photographs taken. They were analysed using Image J software. The glass surface with the fibronectin, demonstrated high density of cells and well spread out in comparison with AC-100, which has significant cell density and spreading out. (Figure1A,1B). In contrast the control surface demonstrated only single cell in the photograph (Figure 1C).

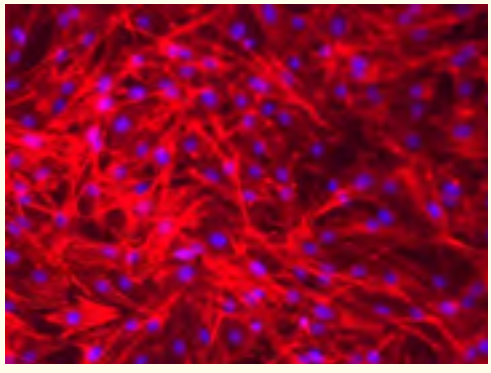

Figure 1A: *Demonstrates that the fibronectin surface has a high density of cells and they are well spread out.*

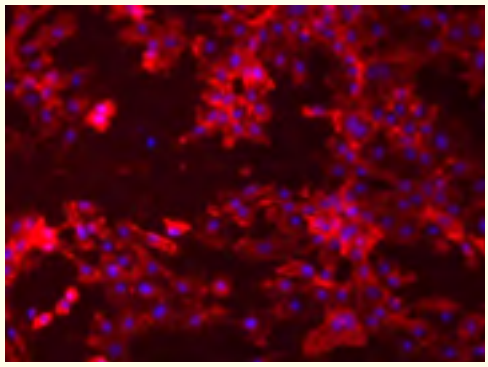

Figure 1B: *The AC-100 peptide surface has a significant level of cell density as well as spreading.*

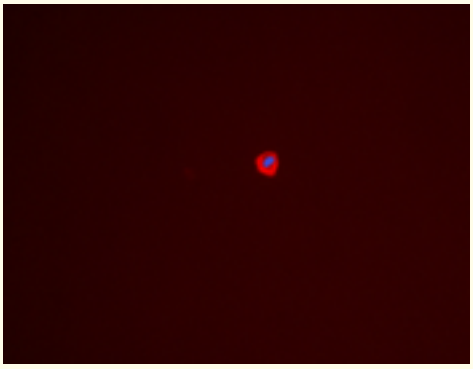

Figure 1C: *The control surface exhibits only one cell can be seen in the image.*

Figure1: *The results of the glass surface cell studies are represented in photographs (A-C).*

The number of osteoblasts that adhered to the AC-100 and FN surfaces were significantly greater than the control surfaces. This pattern is also exhibited on the cell perimeter of the cells analysed per surface.

This was analysed by ANOVA. The cell adherence and perimeter were significantly more in AC-100 slide compared to Control slide (p value < 0.05). The cell adherence and perimeter were also more in the Fibronectin Slide in comparison with control surface (p value < 0.05). However the cell adhesions and perimeter were more in the Fibronectin slide in comparison with the AC-100, were not statistically significant (p value > 0.05) (Figure 2,3).

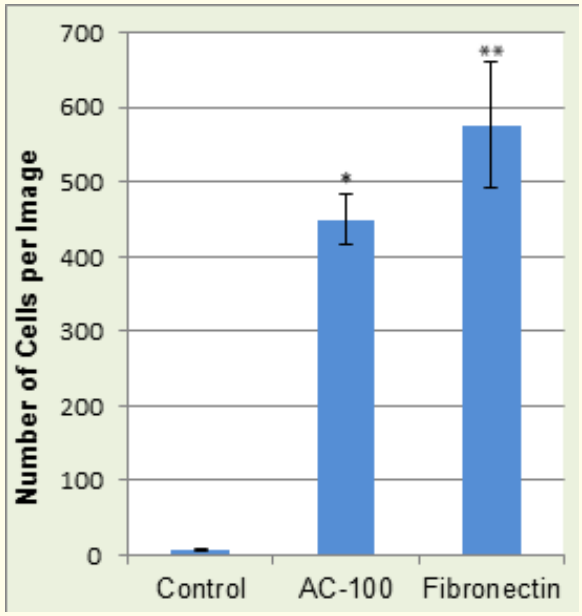

Figure 2: *Shows the average number of cells per image analysed; five random fields were analysed per surface.*

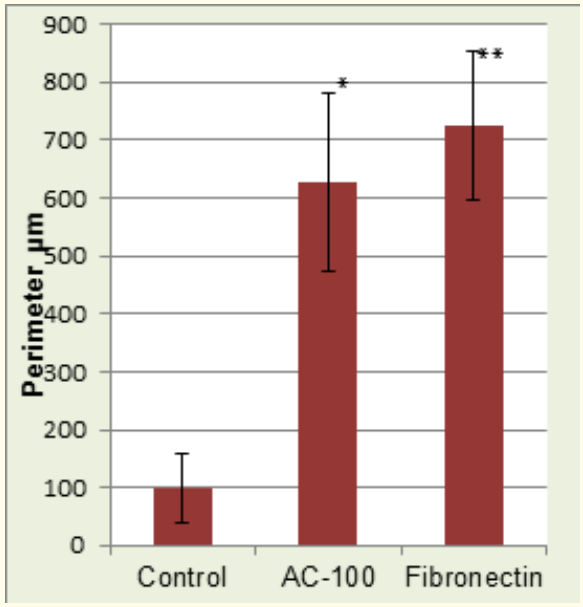

Figure 3: *Shows the average perimeter of twenty random cells which were analysed per surface; the data presented is representative of three independent experiments.*

*Results were analysed by ANOVA, *p < 0.05 compared to "Control," **p < 0.05 compared to "Control."*

Discussion

Fibronectin (FN) is a component of the extracellular matrix allows osteoblasts to adhere to the ECM and form bone [6]. FN provides

this adhesive quality with the RGD domain. RGD allows integrin cell receptors present on the cell membrane of osteoblasts to interact and this provides the intercellular communication required for cell adhesion [7].

The structure of AC-100 contains the RGD motif important for osteoblast adhesion [8-10]. From the published reports it is evident that both the RGD and SGDG motifs need to be present for AC-100 to exhibit its osteogenic behavior [8]. However, the reasons why AC-100 is not as effective at osteoblast adhesion as FN could be related to the RGD motif not being as accessible to osteoblasts as FN's RGD motif. AC-100 has a long amino acid structure with side chains, which may have an effect on the exposure of the RGD motif to the osteoblasts [10]. Another explanation could be low efficiency of the coupling protocol for binding AC-100 to the glass slips.

The control surfaces also showed evidence of osteoblast adhesion even though there weren't any RGD binding sites present on the surface. Osteoblasts that loosely adhere onto the surface could produce fibronectin and other adhesion molecules that aid their binding to the surface [7,11,12]. Therefore, if the cells were allowed to be cultured for a longer period of time, then we should theoretically see a larger number of cells present.

Cell perimeter is a measure of the cell size and also an indirect way of assessing how developed cells are on the surface [10]. Through integrin signalling from the ECM *in-vivo*, osteoblasts are able to develop, replicate and differentiate. Integrin signalling via FN and AC-100 provides an artificial ECM stimulation for the normal cellular processes. The perimeter was measured using Image J software and was open to observe bias. This can be attributed to the presence of the RGD motif on FN and AC-100. On the control surface, we found that the cells are not as developed as the other two surfaces, given the low mean cell perimeter and lack of integrin intercellular signalling.

In this study, it has been demonstrated that the osteogenic character of peptide AC-100 exhibited effective spreading on glass surface with osteoblast adhesion and differentiation. These results are representative of preliminary experiments. The limitations of this research were small sample size, which can influence the significance of the results, future studies could look to increase the sample size. Future work our department is looking to carry out surface studies using titanium to see how effective AC-100 peptide is for osteoblast adhesion.

Ethical Standard

The paper Involves no Human nor animal researches (i.e. review articles, materials tests, etc),thus ethical standards do not apply. The study was approved by the Research Ethics Committee (or Institutional Review Board).

Conflict of Interest

The author (T.M) did receive funding or grants in support of their research for or preparation of this work from Get Orthopaedics.

The study was approved by the Research Ethics Committee.

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