# *In vitro* adhesion and biocompatability of osteoblast-like cells to poly(methylmethacrylate) and poly(ethylmethacrylate) bone cements

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A bone cement, poly(ethylmethacrylate)/*n*-butylmethacrylate (PEMA/nBMA) has been developed with lower exotherm and monomer leaching compared to the traditional poly(methylmethacrylate)/methylmethacrylate (PMMA/MMA) cement. This study compares the *in vitro* biological response to the cements using primary human osteoblast-like cells (HOB). Cell attachment was qualified by immunolocalization of vinculin and actin cytoskeleton, showing more organization on PEMA/nBMA compared to PMMA/MMA. Proliferation was assessed using tritiated thymidine incorporation, and phenotype expression determined by measuring alkaline phosphatase (ALP) activity. An increase in proliferation and ALP activity was observed on PEMA/nBMA compared to PMMA/MMA. The results confirm the biocompatability of PEMA/nBMA, and an enhanced cell attachment and expression of differentiated cell phenotype.


## 1. Introduction

Fixation of bony structures has lead to the development of new biomaterials with a view to enhance the bone prosthesis interface and long-term implant fixation. Charnley and Smith developed poly(methylmethacrylate)/methylmethacrylate (PMMA/MMA) in the 1960s [1] as a bone cement. PMMA is still the current standard for cemented prostheses, providing immediate structural support. The high exotherm produced by the curing of the PMMA cement (up to 100 °C) leads to necrosis of cells apposed to the implant. This cell death contributes to the damage to blood perfusion attributed to intermedullary reaming during implant fixation [2, 3].

Cell necrosis around the implant may also arise from leaching of chemical constituents from the cement; such as methacrylate monomer (approximately 2.0% residual monomer in cement after curing) and N,n-dimethyl-p-toluene (DMT) polymerization accelerator [4, 5].

Mechanical characteristics have been a problem (historically) with PMMA cements; polymers produced by the mixing of the cement phases are usually brittle, and have a poor fatigue life [6]. Futhermore, the most important but least understood factor in long-term stability is the biological response of the host to the biomaterial.

With these factors in mind poly(ethylmethacryalte)/n-butylmethacrylate (PEMA/nBMA) has been developed. This cement system has a low curing exotherm (55 °C), and low levels of monomer leaching. The PEMA system has a relatively low modulus of 700 MPa with a high level of ductility (greater than 50% strain to fracture), and shows better fatigue toughness than PMMA [7, 8].

*In vivo* toxicity and biological evaluation have also shown good biocompatability of the PEMA/nBMA cement [9, 10]. In this study, primary human osteoblast-like (HOB) cells were used to investigate the *in vitro* response of bone cells to PMMA/MMA and PEMA/nBMA cements. *In vitro* systems allow the study of tissue–material interactions without the complexities of *in vivo* models [11]. HOB cells were used to study a normal human bone cell response [12].

The initial attachment of the cells to the materials is necessary for subsequent proliferation, and expression of phenotype. Cells attach via focal adhesion plaques and this interaction is brought about via transmembrane integrin proteins, located at sites of adhesion. This process results in a large number of different cellular responses, depending on the integrin expressed by the cell and the surrounding extracellular matrix (ECM) [13, 14]. ECM proteins, integrins and cytoskeletal

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components are involved in cell adhesion and spreading [15, 16]. Communication between the cell and the extracellular environment occurs by transduction of signals via the actin cytoskeleton from integrin proteins to the cell nucleus [17].

When a material is implanted *in vivo*, it is immediately covered with a thin layer of extracellular fluid, and it is through this layer that the cells interact with the implant material. ECM proteins within the extracellular fluid can become bound to the material, depending on the surface chemistry [18]. The interaction of integrins with ECM constituents such as collagen, fibronectin, vitronectin, thrombospondin, and osteopontin can determine the eventual expression of phenotype [19, 20].

In the present study, visualization of actin cytoskeleton, and vinculin localization at sites of focal contact has been performed, using confocal laser scanning microscopy, to observe cell adhesion on the cements. Proliferation and cell phenotype have been measured biochemically to quantify biocompatibility of the materials.

## 2. Materials and methods

Disks of 1.2 cm diameter PMMA/MMA (supplied by Coripharm GMBH, Germany) and PEMA/nBMA [21] cements were prepared by addition of the monomer to the polymer and stirred, until fully wetted, under controlled temperature conditions ($22 \pm 2\,^{\circ}C$).The prepared disks were sterilized by gamma irradiation at a dose of 2.5 Mrad (Swann Morton, UK) using standard procedures for medical devices.

### 2.1. *In vitro* cell culture

HOB cells were cultured on the materials and control Thermanox (TMX, Life Technologies) at $5 \times 10^6$cells ml$^{-1}$ for 1, 3, 7, 14, and 28 days under conditions described in a previous study [22].

### 2.2. Cell growth, proliferation and differentiation

Cell growth and proliferation were assessed using total DNA and tritiated thymidine ($^3$H-TdR) incorporation, cell phenotype was quantified by biochemical measurement of alkaline phosphatase (ALP). These methods have been described in a previous study [22].

### 2.3. Focal Contact Formation

HOB cells were seeded onto the materials ($5 \times 10^6$cells ml$^{-1}$) and cultured for 72 h. At each point the cells were fixed in 4% formaldehyde/phosphate buffered saline (PBS). When fixed the samples were washed with PBS, and permeabilized using a permeabilizing buffer (10.3 g sucrose, 0.292 g NaCl, 0.06 g $MgCl_2$, 0.476 g Hepes buffer, 0.5 ml Triton X, in 100 ml water, pH 7.2) at 4 °C. The samples were then incubated at 37 °C for 5 min in 1% BSA/PBS, followed by the addition of anti-viculin primary antibody (hVIN-1 (Sigma)) for 1 h (37 °C). The samples were washed in PBS/Tween 20. A secondary FITC conjugated antibody (rabbit anti-mouse, DAKO) was added for 1 h (37 °C). A further wash followed, and the samples were then viewed on a confocal laser scanning microscope (CLSM, Noran).

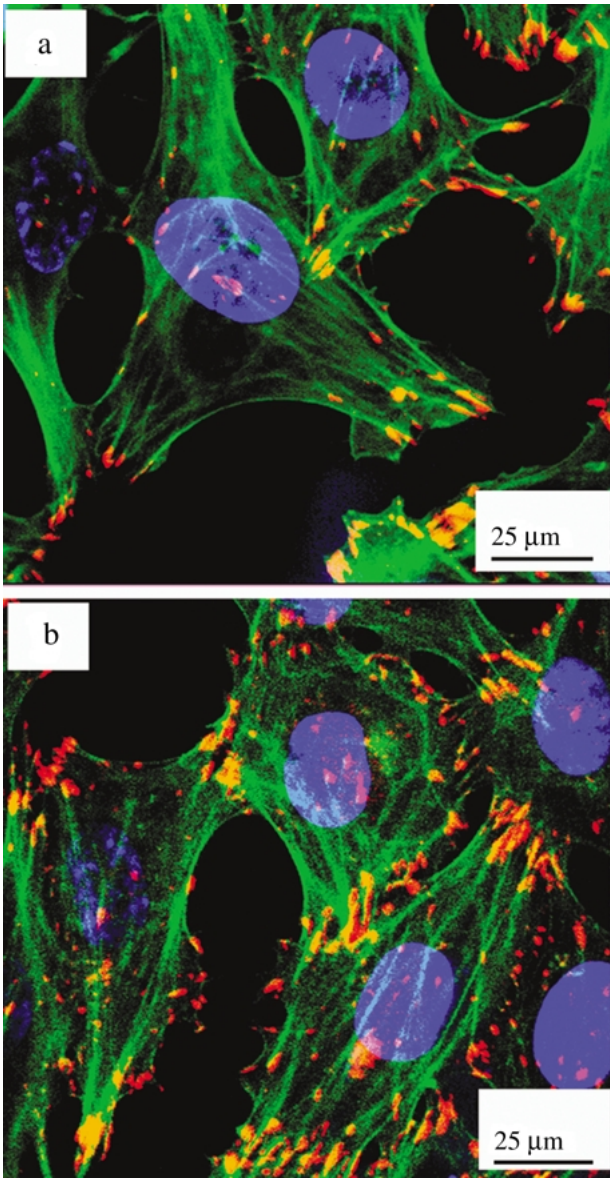


*Figure 1* (a) CLSM image of HOB cells adhering to PMMA/MMA, where blue represents nucleus, green represents actin, red (orange color crossover) represents vinculin adhesion plaques. (b) CLSM image of HOB cells adhering to PEMA/nBMA, where blue represents nucleus, green represents actin, red (orange color crossover) represents vinculin adhesion plaques.

### 2.4. Cytoskeletal organisation

HOB cells were seeded onto the materials ($5 \times 10^6$cells ml$^{-1}$) and cultured for 72 h. At each point the cells were fixed in 4% formaldehyde/phosphate buffered saline (PBS). When fixed, the samples were washed with PBS, and permeabilized using a permeabilizing buffer (10.3 g sucrose, 0.292 g NaCl, 0.06 g $MgCl_2$, 0.476 g Hepes buffer, 0.5 ml Triton X, in 100 ml water, pH 7.2) at 4 °C. The samples were then incubated at 37 °C for 5 min in 1% BSA/PBS, followed by the addition of Phallodin-FITC probe (Sigma) for 1 h (37 °C). The samples were washed in PBS/Tween 20 ($3 \times 5$ min rinses) and viewed by CLSM.

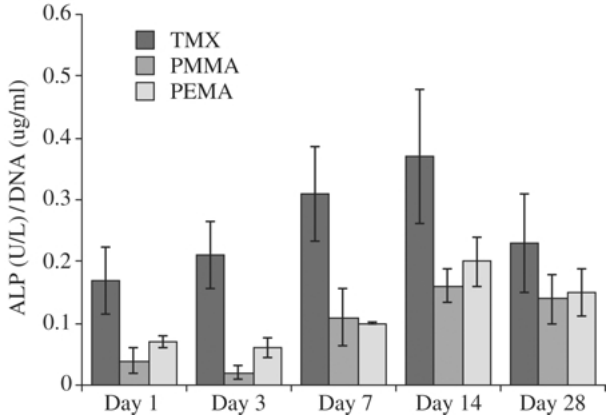


*Figure 2* ALP (U/L)/DNA (μg/ml) for HOB cells on control TMX and the test materials PMMA/MMA and PEMA/nBMA cultured over a 28 day period (results are the mean ( ± SD, $n = 5$).

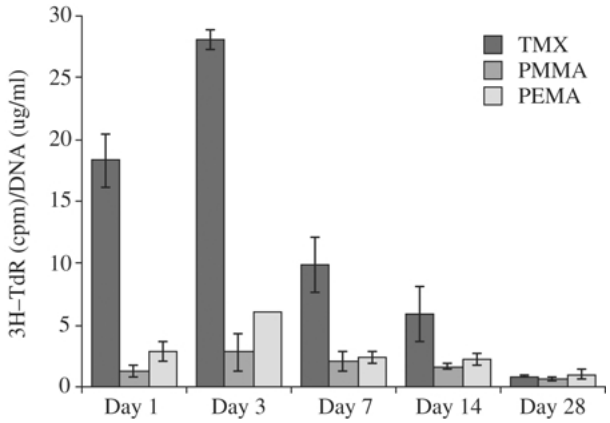


*Figure 3* $^3$H-TdR (cpm)/DNA (μg/ml) for HOB cells on control TMX and the test materials PMMA/MMA and PEMA/nBMA cultured over a 28 day period (results are the mean ( ± SD, $n = 5$).

## 3. Results

A higher expression of focal adhesion plaques viewed by vinculin localization was observed on PEMA/nBMA compared to PMMA/MMA, suggesting higher levels of cellular adhesion to the material (Fig. 1). This result was consistent with the observation of the, generally, more highly organized actin cytoskeleton, which forms stress fibers from adhesion plaques once attached, on PEMA/nBMA as compared to PMMA/MMA (Fig. 1).

It has been suggested that adhesion plaque formation and cytoskeletal organization may be important in cell phenotype expression [19]. The biochemistry results are in agreement with this statement with higher levels of HOB proliferation (Fig. 2) and ALP activity (Fig. 3) observed on PEMA/nBMA compared to PMMA/MMA.

## 4. Discussion

The enhanced expression of adhesion plaques on PEMA/*n*BMA compared to PMMA/MMA suggests higher levels of cellular adhesion. Actin cytoskeleton is connected to adhesion plaques, with a transition from parallel actin bundles, to stress fibers observed with adhesion [13, 14]. The increased organization of actin on PEMA/nBMA reinforces the observation of an increased number of adhesion plaques compared to PMMA/MMA.

The initial attachment of the cell to the material is of importance, because once attached the cell can replace proteins deposited on the material surface with endogenous fibronectin. This process allows further adhesion [23]. Integrins within the adhesion plaques mediate the attachment of cells to extracellular proteins absorbed onto material surfaces [19].

The attachment of cells to material surfaces also affects signal transduction and gene regulation [19, 24]. Thus, the observation of greater proliferation and differentiation consistent with the CLSM vinculin and actin immunolabeling.

## 5. Conclusion

CLSM study of vinculin and actin, and biochemical quantification of ALP activity and proliferation, demonstrate an increased cellular activity on PEMA/nBMA compared to PMMA/MMA.

It is also noted that the event sequence from cell attachment to expression of phenotype appear to be closely linked, and a substrate offering higher potential for adhesion is best suited to allow subsequent differentiation.

Integrins within the adhesion plaques mediate the attachment of cells to extracellular proteins absorbed onto material surfaces [19].

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