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Stephen M. Horowtiz
Allegheny University Hospitals

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MACROPHAGE RESPONSE TO POLYMETHYLMETHACRYLATE PARTICLES

Stephen M. Horowitz

Department of Orthopaedic Surgery, Allegheny University Hospitals, Hahnemann,
219 N. Broad Street, 7th Floor, Philadelphia, PA 19107

Telephone number: (215) 762-4275 / FAX number: (215) 762-1731 / E-mail: horowitzs@auhs.edu

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Abstract

This paper reviews studies performed by this laboratory involving the macrophage response to implant particles. Through the development of a tissue culture model, we have studied the events which occur following macrophage phagocytosis of bone cement particles, the released mediators and cell interactions which may ultimately lead to bone resorption, and the inhibition of this process with the use of pharmaceutical agents. This is divided into the following sections: Histologic response of macrophages to cement particles / toxicity / and mediator release, the effects of particle size and composition on mediator release by macrophages, macrophage / osteoblast interactions, macrophage / osteoblast / osteoclast interactions, the role of tumor necrosis factor alpha in particulate induced bone resorption, mechanisms of cellular recruitment in aseptic loosening, macrophage / osteoblast coculture model, titanium, pharmaceutical inhibition of particulate induced bone resorption, and work by other investigators using the macrophage model.

Key Words: Aseptic loosening, macrophages, particles, cytokines, orthopaedics, implants, materials, polymethylmethacrylate, titanium.

Introduction

Aseptic loosening can be defined as the failure of the bond between implant and bone in the absence of infection. Most investigators currently believe that aseptic loosening is secondary to bone resorption at the interface between implant and bone stimulated by the presence of particulate wear.

When we began working on this project in 1983, very little interest centered on the possible role of particulates in aseptic loosening with most theories involving some type of toxic response to the polymethylmethacrlyate cement (PMMA). Petty [21] had demonstrated the toxic effects of polymethylmethacrylate monomer upon leukocytes. Stauffer [23] suggested that loosening may be a result of bone necrosis at the bone-cement interface secondary to exothermia of polymerization of the cement, or from the physical presence of cement in the bone interstices. Linder and Romanus [19] suggested that resorption at the interface may be secondary to bone necrosis resulting from an impairment of bone vasculature. Pederson [20] demonstrated an inhibitory effect of PMMA monomer upon bone turnover *in vitro* and suggested that monomer may play a role in loosening by inhibiting bone formation at the interface. These theories became less popular when retrieval studies of well fixed implants from cadavers demonstrated little if any evidence of necrotic bone. In addition, further studies on the kinetics of polymethylmethacrylate monomer dissolution demonstrated that within a few hours to at most a few days, no monomer could be detected at the site of polymerization even by the most sensitive methods. Aseptic loosening usually takes several years to occur, and so, it is unlikely that any detectable monomer is present when the interface is converting from a well-fixed to loose histologic appearance.

The early *in vitro* models of the loosening process primarily involved exposing cells to polymethylmethacrylate monomer in tissue culture and then studying the degree to which this monomer was toxic. This evolved from the initial theories in which it was believed that a toxic response to implantation of the PMMA was a

major factor in later loosening.

In the late 1970's and early 1980's, a clearer picture of the loose and well fixed bone cement interfaces were emerging from the retrieval studies. Willert [24] demonstrated a relationship between the presence of small prosthetic particles and what he described as a foreign-body reaction which included a macrophage response in the tissue which surrounds prosthetic joints. This was followed by histologic studies demonstrating an association between macrophage phagocytosis of particles and bone resorption at the loose interface, which led to the theory that the mechanism of loosening may not be a toxic response to cement implantation, but instead an inflammatory response to the production of particulates [2, 3]. The most widely accepted theory at the present time, is that the release of mediators from macrophages engaged in particle phagocytosis stimulates bone-resorption at the bone/implant interface. It is this resorption which disrupts the bond formed between implant and bone and ultimately leads to clinically recognized loosening.

The basis of the *in vitro* model for aseptic loosening which we have been developing, is to more clearly understand the mechanism of this process by bringing the material (particulate) and cellular elements of the interface together in a controlled environment and studying the response.

Research on Macrophage Response to Implant Particles

Histologic response of macrophages to cement particles / toxicity and mediator release

In our first project, we were interested in whether macrophages would phagocytize PMMA particles *in vitro* as they were appearing to do at the interface in the retrieval studies, and also whether phagocytosis of these particles was toxic to these cells. This interest in toxicity arose from prior studies demonstrating the toxicity of the monomer. In our first experiment, rat peritoneal macrophages were exposed to PMMA particles obtained by grinding a block of cement with a drill bit. This simple experiment demonstrated active phagocytosis of these particles by macrophages. In addition, many cells detached from the bottom of the plates and electron microscopy demonstrated these to be dying or dead with PMMA particles within phagosomes.

In the studies which followed, it was demonstrated that exposure to PMMA particles significantly inhibited DNA synthesis and cytotoxic function, and also led to lactate dehydrogenase release reflecting cell injury [12, 13]. This response was not secondary to any soluble contaminants such as monomer, nor was it simply a dose response to the physical presence of particles since it did not occur when the cells were exposed to the same concentration of polystyrene particles. Exposure to both PMMA and polystyrene particles led to release of arachidonic acid metabolites. These studies demonstrated the coexistence of both the toxic and stimulatory effects upon macrophage physiology of PMMA particle phagocytosis [12, 13].

Effects of particle size and composition upon mediator release by macrophages

In our next study, we demonstrated that macrophages which phagocytized particles of cement in tissue culture were similar morphologically to macrophages which phagocytized cement particles at the interface *in vivo*. This response occurred with cement preparations actually used in the operating room, and there was no difference between commercially available bone cement preparations such as Simplex (Howmedica; Rutherford, New Jersey) or Palacos (Richards; Zimmer, Warsaw, Indiana). Phagocytosis of the particles appeared to be an essential component of the response. The release of mediators was a specific response with the major mediator released being tumor necrosis factor alpha (TNF-α) [15].

On the basis of these studies, we proposed that the mechanism of aseptic loosening was a macrophage-mediated response with two components. The first involves recognition of the mechanical failure of the cement mantle, which results from the production of particles small enough too be phagocytized by macrophages (less than fifteen micrometers). This initiates a biologic response that is characterized by a repetitive cycle of particle phagocytosis and cell death with release of certain bone resorbing mediators, one of which is tumor necrosis factor but not prostaglandin E2 (PGE 2) [15].

Macrophage / osteoblast interactions

The previous experiment demonstrated that exposure of macrophages to PMMA particles leads to release of specific mediators one of which is TNF-α, but not PGE 2. PGE 2 is an inflammatory bone resorbing mediator which has been implicated in several studies as being associated with the loosening process [14].

In addition to macrophages, there are other cells present at the interface in aseptic loosening which are capable of PGE 2 production such as osteoblasts. In this study, osteoblasts which were incubated in conditioned medium from macrophages exposed to cement particles small enough to be phagocytized, increased PGE 2 release eighty fold over unexposed osteoblasts. Incubation of osteoblasts in conditioned medium from macrophages exposed to particles too large to be phagocytized, or to bone cement filtrate, did not lead to PGE 2 release. Based on this, we hypothesized that PGE 2 release in aseptic loosening may be originating from osteoblasts in

response to mediators (such as TNF-α) produced by macrophages following PMMA particle phagocytosis, rather than directly from macrophages [16].

Macrophage / osteoblast / osteoclast interactions

The hypothesis derived from the prior studies is that the mechanism of aseptic loosening involves a macrophage / osteoblast / osteoclast process as opposed to a direct macrophage / osteoclast interaction. To test this hypothesis, we exposed isolated osteoclasts to conditioned medium from macrophages incubated with particles, with and without an osteoblast contribution. Incubation of osteoclast precursors in the presence of macrophage / PMMA medium with an osteoblast contribution, increased the number of rat osteoclast precursors which migrated on to a calcified substrate (dentin). In contrast, incubation of the osteoclast precursors with macrophage / PMMA medium without the osteoblast contribution, not only did not stimulate migration to he dentin slice but actually resulted in a decrease in the number of osteoclasts compared to unexposed control.

This study supports the hypothesis that osteoblasts play an important role in macrophage / PMMA osteoclastic bone resorption [22].

Mediator interactions in particulate induced bone resorption

The purpose of this study was to determine whether or not particulate induced bone resorption was occurring by a mechanism which was dependent upon PGE 2 production by cells in bone. Macrophages were exposed to cement particles, and then this conditioned medium was exposed to rat calvarial bones *in vitro*. The macrophage conditioned medium contained increased levels of tumor necrosis factor alpha (TNF-α), but not interleukin 1 (IL-1) or PGE 2. Exposure of this medium to the calvaria led to release of PGE 2 by the calvaria, but not TNF-α or IL-1. At 24, 48, 72, and 96 hours of incubation, the macrophage / cement particle conditioned medium led to the release of both PGE 2 and ^{45}Ca (bone resorption) from the calvaria. To determine if the release of ^{45}Ca was dependent upon PGE 2 production by the cells in bone, the calvaria were incubated with 600 ng/ml of indomethacin (Sigma Chemicals, St. Louis, Missouri) in addition to the macrophage conditioned medium. The addition of indomethacin was effective in inhibiting both PGE 2 and ^{45}Ca release from calvaria, even after 96 hours of exposure to the macrophage conditioned medium [10].

This study demonstrates that macrophage exposure to bone cement particles leads to the release of mediators which stimulate bone resorption at least in part, through a prostaglandin E2 dependent mechanism.

Role of tumor necrosis factor alpha in particulate induced bone resorption

The prior studies have demonstrated the production of tumor necrosis factor alpha (TNF-α) as the most consistent mediator release following PMMA particle exposure. This finding suggests that TNF-α plays a role in particulate induced bone resorption but does not demonstrate a cause and effect relationship. The purpose of this study was to determine the role of TNF-α in bone resorption secondary to mediator release from macrophages exposed to cement particles. Macrophage exposure to PMMA particles over a time course from 30 minutes to 96 hours led to an increase in TNF-α which was initially detected at 30 minutes with a maximum at 48 hours. Incubation with the macrophage / PMMA conditioned medium significantly increased ^{45}Ca release and PGE 2 release by calvaria. To study the role of TNF-α release in bone resorption, the macrophage / PMMA conditioned medium was then pre-incubated with anti-TNF-α antibody prior to exposure to the conditioned medium to the calvaria. This pre-incubation was successful in significantly inhibiting ^{45}Ca release by calvaria ($p < 0.01$), to levels which were not significantly different from unexposed calvaria [1].

This study demonstrates that TNF-α plays a critical role in initiating particulate induced bone resorption. The sequence of events in this model for particulate induced bone resorption appears to be initiated by the production of TNF-α by the macrophage, followed by production of PGE 2 by bone cells which then induces bone resorption.

Mechanisms of cellular recruitment in aseptic loosening

One of the features which differentiates the well fixed from the loose interface, is the influx of macrophages, fibroblasts and osteoclasts. These cells are present in only small numbers in the well fixed interface, but are present in large numbers in the aseptically loose interface. Many investigators have speculated that the recruitment of these cells is an essential component of the loosening process. The purpose of this study was to improve our understanding of the mechanism by which recruitment of these cells into the interface occurs.

TNF-α is known to be capable of stimulating the release of granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin 6 (IL-6) from osteoblasts. Both of these mediators have been demonstrated to be capable of stimulating recruitment of cells such as macrophages, fibroblasts and osteoclasts, into sites of inflammation [9]. The prior studies have demonstrated that TNF-α is consistently released following exposure

of macrophages to PMMA particles, and that this mediator plays an important role in PMMA particulate stimulated bone resorption. In this study, it was demonstrated that incubation of osteoblasts with conditioned medium from macrophages exposed to PMMA particles leads to the release of GM-CSF, IL-6, and PGE 2. In addition, incubation of the macrophage / particle conditioned medium with antibodies to TNF-α prior to osteoblast exposure inhibited release of GM-CSF, IL-6, and PGE 2. This demonstrates that exposure of macrophages to PMMA particles leads to release of TNF-α which then stimulates osteoblasts to produce GM-CSF, IL-6, and PGE 2. This supports a role for TNF-α not only in particulate induced bone resorption at the interface, but also in stimulating the release of secondary mediators from surrounding cells which leads to the recruitment of other cells into the interface that play a role in the loosening process [11].

Macrophage / osteoblast coculture model

At the interface of the loose prosthesis, macrophages, osteoblasts and fibroblasts are all in close proximity. The prior studies evaluated the effects of mediator released from macrophages upon osteoblasts, but did not take into account the potential effects of mediators released from osteoblasts, or other cells present in bone, upon macrophages.

The purpose of this study was to further define the cellular response to titanium (pure) and PMMA particles in aseptic loosening in a more physiologic environment where macrophages and osteoblasts are cultured together. Macrophages and osteoblasts were cocultured to stimulate the environment around an aseptically loose prosthesis. Macrophages were plated on the bottom of six well plates while osteoblasts were plated on culture dish inserts, and placed into the wells with the macrophages. Incubation of macrophages with PMMA in this system led to release of PGE 2, GM-CSF and IL-6. Incubation with titanium led to release of tumor necrosis factor alpha and IL-6. Exposure of calvaria to media from cells exposed to either PMMA or titanium, led to release of ^{45}Ca [8].

This study is consistent with prior results demonstrating release of TNF-α. It also revealed release of other mediators previously identified as playing a role in this process, as well as the stimulation of bone resorption.

Titanium

The purpose of this study was to define the role which titanium debris plays in aseptic loosening. Macrophages exposed to commercially pure titanium (1-3 micrometer) exhibited a mediator profile similar to those exposed to polymethylmethacrylate. This response consisted of increased release of TNF-α but not PGE 2 or IL-1. Osteoblasts increased production of PGE 2 when exposed to media from titanium-stimulated macrophages but not IL-6 or granulocyte macrophage-colony stimulating factor. Media from macrophages exposed to titanium did not lead to bone resorption, as measured by ^{45}Ca release in organ culture. This result is different from the prior studies in which calvaria exposed to media obtained following exposure of macrophages to titanium which were in coculture with osteoblasts, did lead to ^{45}Ca release. The cellular response to titanium is characterized by release of TNF-α from macrophages, and PGE 2 from osteoblasts exposed to the macrophage conditioned medium. A comparison of the results of this study with others involving exposure of macrophages in tissue culture suggests that titanium may not be as inflammatory as other particles in the aseptically loose joint [5].

Pharmaceutical inhibition of particulate induced bone resorption

Pharmacologic inhibition of the pathway leading to bone resorption offers the potential for improving implant longevity. In this study, a rat calvaria / macrophage coculture model was used to study the effects of various agents upon bone resorption induced by macrophage exposure to bone cement particles. The experimental group consisted of calvaria bone disks set in tissue culture medium on stainless steel platforms into wells with macrophages adherent to the bottom which are exposed to the particles. This was compared to the opposite (control) side calvaria in which the wells did not contain macrophages. The medium from this system was first assayed to ^{45}Ca, TNF-α, interleukin 1-alpha (IL-1α), and PGE 2 release. The system was then challenged with indomethacin, anti-TNF antibody, calcitonin (Rorer Pharmaceuticals, Fort Washington, PA) and pamidronate (Ciba Pharmaceuticals, Summit, NJ).

TNF-α, PGE 2, and ^{45}Ca were released in significant amounts in this system when compared to control. IL-1α was not detected in this system. Indomethacin inhibited the production of PGE 2, but did not affect TNF-α release or inhibit release of ^{45}Ca. Anti-TNF antibody neutralized the presence of TNF-α to undetectable levels, but did not affect PGE 2 release or inhibit ^{45}Ca release. Addition of calcitonin did not inhibit ^{45}Ca release by calvaria. In contrast, addition of pamidronate was effective in inhibiting release of ^{45}Ca even after 96 hours of incubation.

In the prior studies using the same cell line and particle preparation but with a "one-way" model, bone resorption stimulated by incubating calvaria with media obtained from macrophages exposed to particles was dependent upon TNF-α and PGE 2 release. In the "two-way" model, in which macrophages are incubated with

particles in the same well as the calvaria, this relationship no longer exists. In this system, pamidronate was the only agent tested which suppressed the increase in bone resorption associated with the macrophage exposure to bone cement particles to levels which were not significantly different than unexposed calvaria. This suggests that agents which act late in the pathway leading to bone resorption may be more effective than those which work by inhibiting a particular mediator earlier in the pathway [17].

The findings from this study demonstrate that pamidronate, or other bisphosphonates, may be effective in inhibiting bone resorption at the implant / bone interface in association with the macrophage response to implant particulates.

Work by Other Investigators

In general, our studies are in agreement with other investigators as they have also concluded that release of cytokines is a crucial component in the mechanism of aseptic loosening. One area of difference in our results relates to the production of interleukin 1-beta (IL-1ß). Other investigators have found IL-1ß release following particle exposure in greater amount than were detected in our studies [6]. These differences may be secondary to use of different cell lines, conditions of exposure, or particle preparations. In our experiments, we have only found small amounts of this mediator produced, with TNF-α being by far the mediator released most consistently, in greatest amounts, and upon which the response of osteoblasts, and bone resorption is most dependent.

The work in our laboratory has primarily focused on the role of macrophages / osteoblasts / and osteoclasts in the mechanism of aseptic loosening. Work by other investigators have attempted to determine if other cell types such as lymphocytes are involved, and if there is evidence of an immunologic type response. These investigations have involved examining the interface from loose prostheses for lymphocytes, as well as implanting particles into animals with and without immunologic deficiencies and studying the immunologic response. Although there is some controversy, most of the studies indicate that the role of lymphocytes in aseptic loosening is minimal [4, 7, 18].

Conclusions

These studies help define the response of macrophages to implant particles. The results from these experiments indicates that the biologic response is probably initiated when prosthetic wear particles less than 15 micrometers are formed. Macrophage phagocytosis of these particles stimulates the release of inflammatory mediators one of which is TNF-α. The particles are ultimately toxic to the macrophage as reflected by inhibition of DNA synthesis, inhibition of cytotoxic function and release of lactate dehydrogenase. Ultimately the cell dies with release of the particles for phagocytosis by another macrophage. The TNF-α which is released by the macrophage stimulates osteoblasts to produce granulocyte macrophage colony stimulating factor and IL-6, both of which stimulate recruitment of additional macrophages, osteoclasts, and other inflammatory cells into the area of particle accumulation at the interface. TNF-α also stimulates PGE 2 release by the osteoblasts which may have an effect on down regulating further production of TNF-α and also in stimulating osteoclastic bone resorption. This bone resorption disrupts the bond formed between implant and bone, and leads to clinically recognized aseptic loosening.

The demonstration that the bone resorption stimulated by macrophage exposure to these particles can be inhibited by a bisphosphonate, offers the hope that these agents may one day play a role clinically in treating patients who have aseptic loosening of their prosthetic reconstructions.

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Discussion with Reviewers

J.L. Saffar: Why do some macrophages die after phagocytosis of PMMA particles? Does macrophage death contribute to the clinical and in-vitro features, for instance by releasing mediators stored in the cells, e.g., proteolytic enzymes?

Author: This question raises several interesting points. We have found PMMA particles to be toxic to macrophages as reflected by several parameters including release of intracellular LDH (lactate dehydrogenase) and inhibition of DNA synthesis [12, 13]. These changes were not seen when macrophages were exposed to styrene particles when the effects of particle size, number and shape were controlled for. What the mechanism is by which PMMA induces its' toxic effect is unclear but would make an interesting study.

Macrophage death probably contributes to the clinical picture of loosening by releasing particles which can then stimulate other macrophages to produce inflammatory mediators. In this way, a small amount of particles can induce a prolonged response. The mediators which we have studied appear to be synthesized by the cell in response to particle exposure. This can be seen by increases in the amount of mRNA for TNF-α which is produced within the cell, in response to particle exposure. We have not studied proteolytic enzymes.

J.L. Saffar: How can the author explain the discrepancy in mediator release after PMMA or titanium exposure? In particular, why, and how osteoblasts exposed to medium containing large amounts of TNF-α respond differently depending on the activating particle? This strongly suggests that macrophages have released simultaneously other mediators modulating the osteoblast response. Has the author explored this possibility?

Author: It is unclear to us exactly why there is a difference in mediator release between PMMA and titanium exposure. In general, why some materials tend to be more inflammatory than others is an interesting and important question. We speculate that it may relate to differences in the toxicity of the materials, and the effects of this toxicity upon the synthesis of the inflammatory mediators stimulated by these particles. We are currently exploring this question.

The difference in the osteoblast response to conditioned medium from macrophages exposed to PMMA and titanium is probably related to the amount of cytokines in the media. The mediator profile between these two materials when exposed to the J774 macrophage cell line was the same for all the mediators we tested, but

there were some differences in the amounts as has been pointed out. We have found small amounts of IL-1ß which may be acting synergistically to enhance the effect of the TNF-α in the media taken from macrophages exposed to both PMMA or titanium. There is a possibility that some mediators which we have not assayed for may also be playing a role. Since the osteoblast response was inhibited by treatment with antibodies to TNF-α, it would be our opinion that these mediators are probably working more to enhance the effects of TNF-α rather than by stimulating the osteoblasts directly.

N.P. Ziats: The role of inflammatory cytokines are important with regards to biocompatibility. Dr. Horowitz's group has implicated TNF-α as an important mediator but Glant and co-workers (Glant and Jacobs [6]; Shanbhag *et al.* [26]), as well as those studying inflammatory processes with dental materials, have shown significant levels of IL-1α or IL-1ß production. However, as Dr. Horowitz states, there are differences with regard to cell types and conditions. Three questions are important. One, is the heterogeneity of the patient population so significant that differences seen cannot be attributed to isolation of cells or assay technique? Is there any evidence that cells from the interface (not peripheral blood monocytes), i.e., isolated from the aseptic loosening sites in human or animal implants, produce these factors? Does TNF-α stimulate the migration of other macrophages or other cells to these implant sites?
Author: Immortalized cells were used in our study, and was one of the cell types used in the study by Glant and Jacobs [6]. Isolation techniques would be more important in comparisons involving human monocytes. It has been our experience (unpublished) that the mediator profiles between the immortalized cells and isolated monocytes are usually similar although there frequently can be differences in the amounts of mediators being released. There are some differences between immortalized lines as well. For example, some cell lines tend to produce more TNF-α and others tend to produce more IL-1ß given the same stimulus.

We have found IL-1ß release, but in much lower levels than TNF-α (unpublished observation). It should be noted that in the studies by Glant and Jacobs [6], TNF-α levels were not determined. In their studies, addition of antibodies to IL-1ß, to conditioned media from macrophages exposed to particles, did not significantly inhibit particulate induced bone resorption. This is in contrast to our studies, where addition of antibodies to TNF-α to conditioned medium from macrophages exposed to particles, did inhibit particulate induced bone resorption [1, 6].

Macrophages at the interface are the main cell type involved in particle phagocytosis. *In situ* hybridization studies have demonstrated expression of RNA for IL-1α and IL-1ß, as well as TNF-α in macrophages at the interface. I am not aware of any studies demonstrating production of TNF-α or IL-1α or IL-1ß by cell types other than macrophages at the interface. TNF-α probably does stimulate migration of other cells into the implant site, but probably through a second mediator such as GM-CSF or IL-6 [11].

N.P. Ziats: Are cell adhesion molecules upregulated in cells isolated from patients with implants?
Author: We know of no studies which have examined cell adhesion molecules in patients with implants.

N.P. Ziats: Is there any evidence for matrix metalloproteinase production by macrophages (or other cell types) in response to particles *in vitro* or *in vivo*?
Author: We have not studied matrix metalloproteinase activity. Information on this in the setting of aseptic loosening is minimal although there is some evidence that this may be playing a role. One reference for this would be Takagi [27].

H. Plenk: PMMA-bone cement cured during operation contains at least 3% residual MMA (monomer) which can then leach out during the whole implantation period, even more so when the bulk material is separated into wear particles. Also, the leaching of the starter substance dimethyl paratoluidine has been demonstrated (in the German literature [25]); this substance is obviously toxic and was held responsible for adverse tissue reactions. How can the author state that there is no explanation for the obviously toxic effect of PMMA particles *in vitro* and *in vivo*, and that the cytotoxic response is not secondary to any soluble contaminants?
Author: In our experiments on particle toxicity [12, 13], we used a "filtrate" control to control for the presence of soluble toxic contaminants. PMMA particles were suspended in media and then filtered out with a 0.2 micrometer filter. The resulting filtrate was then exposed to the macrophages and its' effects on toxicity studied as an additional control. We would expect that any monomer or starter substance present in the media would also be present in the filtrate [12, 13].

In our experiments [12, 13, 15], we have used PMMA particles manufactured for use in research, crushed polymerized cement from various manufacturers, and the prepolymerized powder (prior to being mixed with the monomer) supplied by the manufacturer for use in the operating room. All these preparations have been demonstrated to be toxic.

In our earlier experiments [12, 13], we compared PMMA and polystyrene particles which were manufactured for use in research. These particles were the same

size and shape, and were exposed to macrophages at the same concentrations. It was found that exposure to the PMMA particles was toxic but that exposure to the polystyrene particles was not. Both particles led to inflammatory mediator release. Exactly why PMMA particles are toxic but the styrene particles are not is unclear. I was not aware of any studies which have answered this question.

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