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Foreign body giant cells in the foreign body reaction to implanted biomaterials, a systematic review

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Abstract

Foreign body giant cells (FBGC) are important cells in the foreign body response (FBR), as they are known to be able to degenerate biomaterials, by oxidation, hydrolysis and MMP secretion. It remains difficult to discriminate between FBGC and other giant cells such as osteoclasts, therefore several established markers will be discussed. Also several targets for the inhibition of FBGC will be discussed as this migth prove to become clinically relevant in order to suppress biomaterial degeneration by FBGC. The reaction of FBGC to several different biomaterials will be discussed as well as there are no reviews discussing this elaborately. Also the comparison of FBGC to mononuclear macrophages was made. The review concludes that FBGC formation varies between different species of animals, different biomaterials, different compositions of the same biomaterial, different surfaces of the biomaterial, as well as different sites of implantation of the biomaterial. Contreversial results have been found with regard to differences between FBGC and mononuclear macrophages with regard to phagocytosis and the secretion of degerative enzymes. However in gerenal mononuclear macrophages seem to display phagocytosis whereas FBGC are found to surround the biomaterial. FBGC were found to be able to secrete degenerative enzymes, cytokines, chemokines, ECM, and angiogenic mediators.

Introduction

One of the first contributions to the field of biomaterials was the implantation of a lens by Harold Ridley in 1949 [1]. Since then the field has grown tremendously, with the global market of biomaterials worth an estimated 150–200 billion US dollar in 2012. The largest markets include the US, Japan, Germany, France, Italy, UK, Brazil, China, Canada and Spain [2].

However, with the invention of biomaterials the arms race against the response of the host to the biomaterial began. This response of the host to the implanted biomaterial has been revered to as the foreign body reaction (FBR). The FBR includes encapsulation and calcification of the biomaterial, and can even lead to device failure [3]. The FBR has been described in detail numerous times, one of these description has been provided by Anderson [4]. The FBR will be explained in further detail later on in this review.

Countless different biomaterials have been used, of which metals, ceramics and polymers form the main groups [5]. Modifications are made constantly to these biomaterials in order to reduce the FBR to the material and therefore lower the risk of device failure.

One of the notable cells involved in the FBR are macrophages. Macrophages can be divided in two main groups being the M1 macrophages and the M2 macrophages, these are involved in inflammation and tissue repair, respectively [6, 7]. Macrophages display high plasticity in their reaction, depending on their micro-environment and its stimuli [6]. One of the main functions of macrophages is phagocytosis, however upon encountering a biomaterial they have the ability to fuse and form foreign body giant cells (FBGC), a concept known as frustrated phagocytosis [8]. However fusion of macrophages has also been found under phagocytic circumstances, so the term frustrated phagocytosis is a bit controversial [8].

These FBGC are considered to be a trademark of the FBR and are associated with biomaterial degeneration, for example by surface cracking of polyether urethane (PU) [9]. FBGC have been found at the tissue-material interface of biomaterials [10]. And FBGC are capable of being present on to the biomaterial for the entire lifetime of the biomaterial [11, 12]. They have been reported to cover around 25% of the biomaterial and grow up to 1 mm in diameter, consisting of hundreds of nuclei [9].

These FBGC are known to derive from macrophages during the inflammatory response of the FBR to an implanted biomaterial [4]. FBGC have been described as early as the 1930's to derive from macrophages, and are known to be able to secrete matrix metalloproteinases (MMPs) [4, 13, 14]. These MMPs degenerate the biomaterial [15, 16]. Since FBGC fulfil a significant role in the degeneration of biomaterials during the FBR, it is important to understand their role in the FBR and the mechanisms by which they degenerate biomaterials.

The role of these FBGC in the FBR and their reaction to different biomaterials remains relatively unclear. This reviews aims to provide an overview of what is known about the reaction of FBGC to different biomaterials as well as their overall role in the FBR. Possible targets of FBGC to reduce their activity in the FBR will also be discussed. This will ultimately provide valuable insights for the biomaterial field, as how to possibly reduce the degeneration of biomaterials by FBGC.

Foreign body reaction

Implantation of medical devices or implants trigger a variety of reactions from the host. Together these reactions are known as the FBR [17]. Anderson provides an overview of the FBR consisting of the following events: acute inflammation, chronic inflammation, granulation of the tissue, fibrous capsule formation and the formation of foreign body giant cells (FBGC) (Fig. 2) [4].

The first step after implantation of a biomaterial is the accumulation of proteins on the surface of the biomaterial [18]. Upon interaction with blood of the host primarily fibrinogen, fibronectin, haemoglobin immunoglobulin G, (IgG), and albumin will absorb to the surface of the biomaterial. Subsequently these proteins will be replaced by bigger molecules such as kininogen and factor XII, this effect is referred to as the Vroman effect [19, 20, 21]. The composition and structure of this protein layer is believed to affect the surrounding tissue, and is even associated with the induction of the FBR because in normal wound healing this protein layer has not been observed [1, 22].

The next step is acute inflammation, which is characterised by neutrophil (polymorphonuclear leukocytes, PMNs) migration into the wound (Fig. 1) [23]. This acute inflammation response to biomaterials is known to be mediated by histamine release and fibrinogen adsorption [24, 25, 26]. Masts cells also regulate this acute inflammatory response [26]. Several biomaterial coatings such as heparin have shown to reduce the acute inflammatory response, but only short term [27].

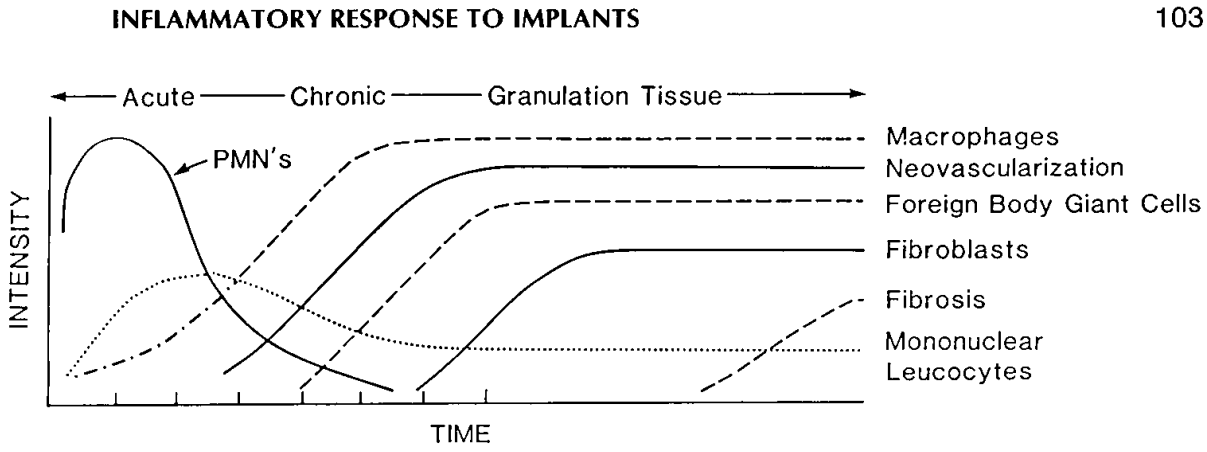

Figure 1: overview over time of the peaks of the different cells during the inflammatory response to implants [23].

Ultimately, the acute inflammatory response is followed by a chronic inflammatory response [17]. During this chronic inflammatory response macrophages, monocytes, and lymphocytes are present (Fig. 1) [17].

In the last stage fibroblasts produce collagen and proteoglycans [17]. There is a pronounced role for macrophages in this process since macrophages can secrete CCL18 which stimulates fibroblast proliferation and collagen production [28]. This deposition of collagen on the biomaterial eventually forms a fibrous capsule, this is known as encapsulation (Fig 2.) [29]. The thickness and degree of degradation of this fibrous capsule varies with implantation site [30]. This encapsulation has resulted in biosensor failure and is therefore one of the major challenges for biomedical engineers [31]. As a result, countless modifications to biosensors have been made to increase their biocompatibility, including changes in texture and porosity of the biosensor, which have been shown to affect the thickness of the collagen capsule [31].

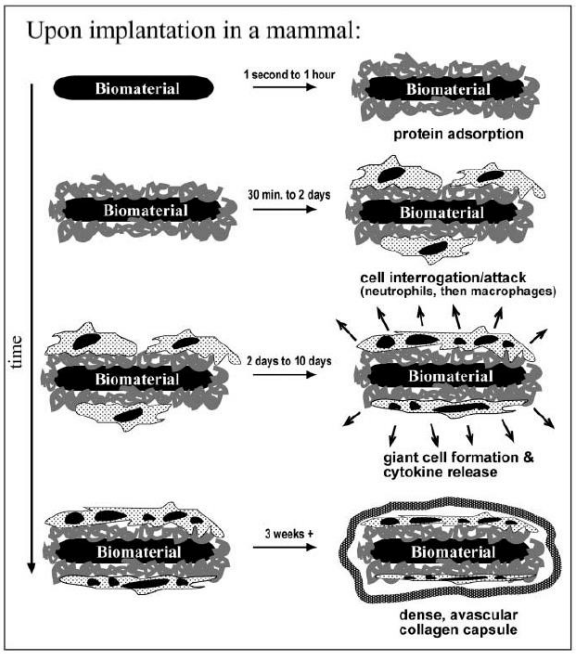

Figure 2: overview of the foreign body reaction upon implantation of a biomaterial over time [1].

Other giant cells

It is of importance to note that there are other giant cells that also derive from macrophages, other than FBGC. The most common one of these are the osteoclasts, which are involved in bone resorption and remodelling [10]. The second giant cell type is the Langhans' giant cells, which are found in granulomatous inflammations. These cells are characterised by a smaller amount of nuclei than FBGC, as Langhans giant cells typically have less than 20 nuclei [10]. The third type of giant cells are the mycobacterium-induced granulomas, which are found in for example mycobacterium tuberculosis [32]. The last type of giant cell is the bone tumor giant cell [32]. Giant cells have been reported in other diseases than bone tumors as well. For example there are giant cells that are associated with spinal cord injury, in a rat model giant cells with a diameter of more than 50µm appeared after 5 days post injury [33]. Another condition in which multinucleated macrophages participate is giant cell arteritis, which is characterised by intimal hyperplasia and luminal obstruction [34]. Multinucleated macrophages have also been found in Crohn's disease (CD) [35]. In which macrophages displayed a fusion index of 22 to 35 percent and a number of nuclei of 11 to 28 after 3 days of incubation [35].

There are also diseases in which giant cells participate that derive from cells other than macrophages. For example the multinucleation of epithelial cells caused by viral infection or cell cycle dysregulation. These multinucleated epithelial cells have been reported in several organs, such as the duodenum, and lead to abnormalities in epithelial growth, development and differentiation [36]. Fibroblasts have also been reported to form multinucleated cells [37]. Myofibroblasts also displayed signs of fusion, indication a role for multinucleated fibroblasts in fibrosis [38]. Fibroblast multinucleation is also associated with aging of the periodontal ligaments [39].

Although each of these giant cells will provide an interesting subject of study, this review will only focus on the giant cells involved in the foreign body response, the FBGC.

FBGC formation

During the acute inflammatory response binding of protein ligands such as fibrinogen, fibronectin, vitronectin, IgG and complement fragment iC3b to β2 integrin results in monocyte adhesion [4, 32]. These monocytes express different membrane receptors important in fusion of macrophages to form foreign body giant cells (FBGC) including the mannose receptor, β1 integrin and CCl2 [40, 41].

Apart from the signals from monocytes, macrophages rely on both exogenous factors and endogenous signals which make them competent to fuse [42]. T lymphocytes secrete Il 4 and Il 13, these are well known exogenous factors that stimulate macrophage fusion [4, 42]. Il 4 and Il 13 in turn activate STAT 6 which in turn inhibits STAT 1 via JAK 1 and JAK 3 (Fig. 3) [43]. STAT 1 inhibition results in the expression of dendritic cell-specific transmembrane protein (DC-STAMP) which is essential for macrophage fusion (Fig. 3) [43, 44]. Granulocyte macrophage colony stimulating factor (GM-CSF) has also been found to induce macrophage fusion (Fig. 3) [42].

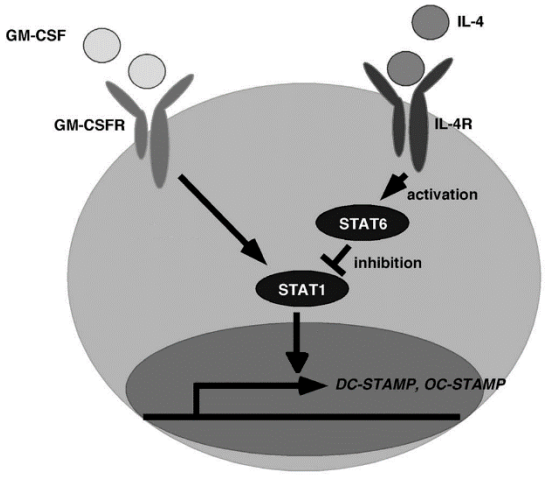

Figure 3: Induction of macrophage fusion by Il4 via STAT 6 and STAT 1. And GM-CSF via STAT 1, resulting in DC-STAMP expression, which leads to macrophage fusion [43].

E-cadherin expression has also been found to be important in the formation of FBGC via IL 4 [32] Also meltrin-alpha has been reported to be involved in FBGC formation [45]. Antioxidant vitamin E (90% α-tocopherol), Con A, PHA, IFN-y, 1α25-Dihydroxyvitamin D3, fibronectin MFR, Dextran sulfate and MMP9 have also been reported to induce macrophage fusion [46, 47, 48, 49, 50, 51, 52, 53, 54].

One of the endogenous signals that derives from macrophages is the transmembrane protein signaling adaptor DNAX activating protein 12 (DAP12). DAP12 stimulates macrophage fusion [42, 55]. Another endogenous signal is the expression of the SHPS-1 receptor by macrophages [40]. Also the expression of CD44 by macrophages is correlated with macrophage fusion [56]. Also CD9, CD36, CD44, CD47 and CD81 have been associated with macrophage fusion [43, 57, 58, 59].

Markers of FBGC

Besides numerous factors that induce FBGC formation, there are also quite a few markers that FBGC express, that have been reported. These markers could be important in correctly identifying multinucleated macrophages. Since it has been stated in the literature that it is difficult differentiating multinucleated giant cells from for example osteoclasts which also derive from macrophages [42, 60]. It is important to note that there is a distinct difference in function as FBGC are unable to resorb bone whereas this is the main function of osteoclasts [47]. To make discriminating between the two even more difficult, TRAP and the vitronectin receptor have been reported as a marker for osteoclasts but have also been found to be expressed by multinucleated macrophages [60, 61]. However there are also studies that report that TRAP is not expressed by FBGC [62]. So it still is rather unclear which markers can actually be referred to as FBGC specific. Or even whether there is an plasticity in the expression of markers by FBGC leading to these opposing results.

However several markers for FBGC have been reported that could help correctly identify multinucleated giant cells. There has been found that Na-K-adenosinetriphosphatases (Na-K-ATPases) are expressed on the plasma membranes of both FBGC as well as osteoclasts [63]. However on the plasma membrane of FBGC the location of these Na-K-ATPases is at the non-adhesive side of the cell [63]. This suggest a functionality in polarisation of the position of these Na-K-ATPases [63]. FBGC also express a high number of calcitonin receptors, which are also believed to be involved in the regulation of local immune reactions [63].

Furthermore McNally et al found that IL4 stimulated FBGC strongly express HLA-DR, CD98, CD86, and B7-H1 (PD-L1) [62]. They also express αX integrin (CD11c), CD68, and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) [62]. According to McNally et al, these molecules might provide interactions with lymphocytes, such as PD-L1, which is believed to be able to down regulate lymphocytes when it binds to its co-receptor on lymphocytes [62]. Suggesting a regulatory role for FBGC.

Ym1 and ALOX15 are two markers of M2 macrophages, these markers were found to be significantly higher expressed in FBGC than in M2 macrophages or osteoclasts, on both mRNA and protein levels [47].

Reaction of FBGC to biomaterials

To my knowledge there are no reviews describing the differences in the response of FBGC to different biomaterials. In this section the reaction of FBGC to several biomaterials will be discussed as this reaction can vary between different biomaterials as well as different implantation sites and implantation techniques [30]. An extensive overview of the differences in the reaction of FBGC to different biomaterials could therefore prove to be important.

Effect of implantation site

Bakker et al studied the effect of implantation site on FBGC in male Wistar rats. At implant sites containing bone and muscle tissue they found multinucleated cells of up to 20 nuclei after the first week of implantation, the number of nuclei increased to around 200 after 13 weeks of implantation. In comparison after 1 week of implantation, tympanic membrane and submucosal implants demonstrated FBGC with a maximum of 10 nuclei. For the submucosal implants this number increased to 30 nuclei after 13 weeks of implantation. And after 13 weeks of implantation the tympanic membrane implants the giant cells did not display more than 20 nuclei [30].

Luttikhuizen et al, showed that this difference in FBR on different implantation sites correlates to cytokine and MMP expression in mice [64]. Degeneration of the biomaterial and leukocyte migration were higher in supra-epicardially implanted collagen when compared to subcutaneously implanted collagen. This difference correlated with the expression of cytokines, including higher expression of IL-1 and IL-6 but lower expression of IL-10 creating a predominantly inflammatory milieu for supra-epicardially implanted collagen. Supra-epicardially implanted collagen also displayed higher expression CXCL1/KC and CXCL2/MIP2 resulting in PMN migration. Gene expression of collagen degrading MMPs did not differ significantly between the two sites of implantation, however MMP activity was higher on supra-epicardially implanted collagen. The expression of MMP9, which is known to attract macrophage and induce fusion, was also higher supra-epicardially [64].

Effect of the surface of biomaterials on FBGC

Anderson states that in general rough or porous implants contain a higher amounts of FBGC than flat surfaces such as those of breast implants where only a one- to two-cell layer of macrophages and FBGC is found [10, 65]. However Anderson presents no data to verifies this statement, so one has to be sceptical about his statements.

Taylor et al do show that rough surfaces of premium electrical grade Teflon (PTFE) display higher macrophage adhesion than smooth surfaces (Fig. 4). Resulting in higher formation of FBGC on the textured PTFE and higher secretion of acid phosphatase, than on the smooth surface. But the fibrous capsule is reduced in thickness on the textured surface when compared to the smooth surface [66].

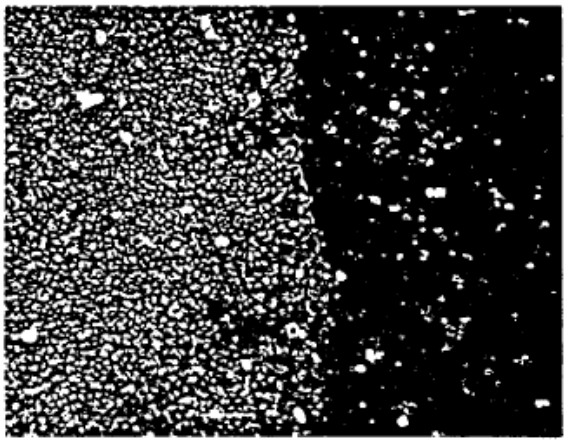

Figure 4:Macrophage adhesion on textured (left) and smooth (right) premium electrical grade eflon (PTFE) surface after 3 days of implantation (160X) [66].

Effect of species

It is also known that the FBR differs between species. In a comparative study it was discovered that mice display less phagocytosis but more calcification of implanted biomaterial, when compared to rats [67]. These differences between different species should be taken into account when researching the FBR in model species.

Glass substrates

In a study performed by Jenney et al, molecules of the alkyl family were bound to silane-modified glass in order to investigate their FBGC formation properties. These molecules ranged from methyl (C1) till octadecyl (C18) (Fig. 5) [68].

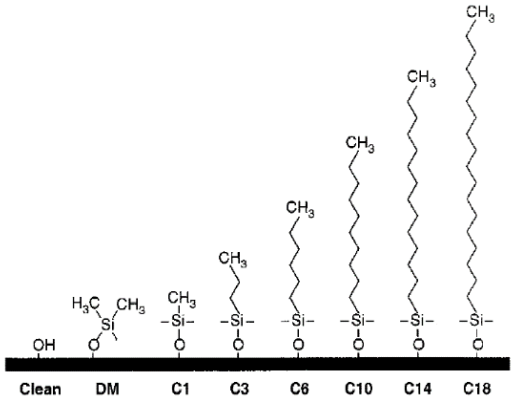

Figure 5: overview of the alkylsilane-modified glass substrates (C1, C3, C6, C10, C14 and C18) including clean glass and silane-modified glass(DM) [68].

There was found that clean glass substrates display low FBGC formation and density. The tetradecyl (C14) and octadecyl (C18) showed a decrease in cell density over the course of 10 days, resulting in a low cell density on day 10 and therefore low FBGC formation (Fig. 6) .

In comparison the silane-modified glass and silane-modified glass containing methyl, propyl, hexyl or decyl displayed high FBGC formation and density (Fig. 6) [68]. This effect was found both for unstimulated monocyte derived FBGC as well as IL4 induced monocyte derived FBGC [68].

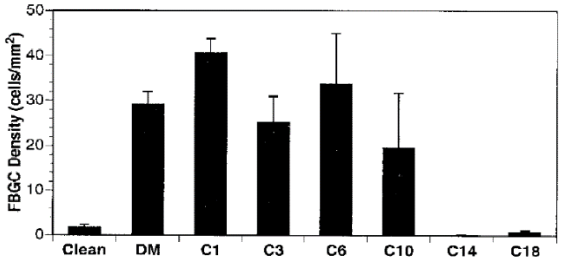

Figure 6: density of IL4 induced FBGC on the alkylsilane-modified glass substrates after 10 days of culturing [68].

Similar to the study on alkyl silane modified glass, they also performed a study with polyethylene oxide (PEO) coupled to silane modified glass. Once again different surfaces were compared, of which the first one is clean glass. Second silanated glass coverslips with 3-aminopropyltrimethoxysilane were used which form an aminopropyl (AP). The third substrate is the activated form of the AP surface by binding of 2,4-toluene diisocyanate (TDI), forming a urea bond. When polyethylene oxide (PEO) binds to the TDI an urethane bond is formed. PEO's with different molecular weights can be coupled using this system, the weights that were used in this experiment were 200, 600, 2000, 4600, and 18,500 Da [69].

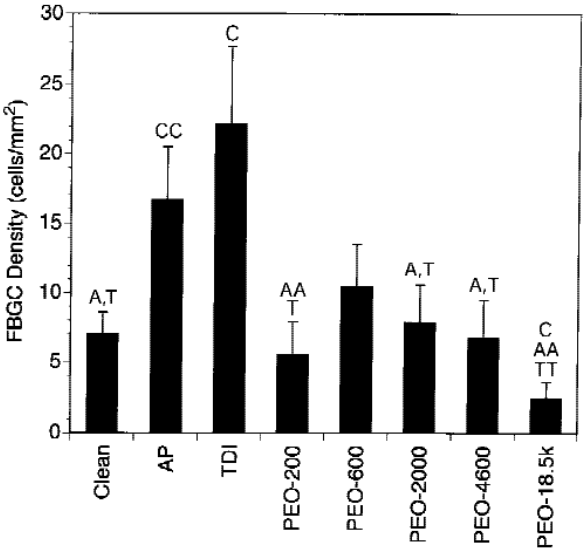

Figure 7: FBGC density (cells/mm^2) of day 10 monocyte cultures treated with IL-4 to induce FBGC formation. On clean glass substrate, aminopropyl modified glass (AP) substrate, 2,4-toluene diisocyanate modified glass (TDI) substrate, and polyethylene oxide modified glass (PEO) of 200, 600, 2000, 4600, and 18,500 Da. Letter codes A,C,T, indicates significant difference to clean glass, AP, and TDI surfaces, respectively. With a single letter being a significance levels of $p < 0.05$. And double letters being a significance level of $p < 0.01$. Mean values and standard errors are shown, $n=4$ [69].

There was found that both the AP and TDI modified surfaces displayed significant increase in FBGC formation compared to clean glass (Fig. 7). This result is unsurprisingly, since clean glass is known to display low FBGC formation [68]. The PEO surfaces of 200, 600, 2000, 4600 Da displayed FBGC formation significantly lower than the AP and TDI modified surfaces, and not significantly different than FBGC formation on the clean glass surface (Fig. 7). So PEO surfaces result in low FBGC formation. The most striking result is the PEO of 18500 Da, since it is significantly lower than AP, TDI and the clean glass surfaces (Fig. 7) [69]. This result is similar to the results of the alkylsilane modified glass surface study where they also found that the longest chain had the lowest formation of FBGC [68]. However in that study this substrate also showed lower cell adhesion, which explained the lower FBGC formation. In this study however the 18.500 Da displayed cell adhesion which only differed significantly from the AT and the TDI surfaces (Fig. 8). The cell density did not significantly differ from the clean glass surface but the FBGC formation was significantly lower (Fig. 8). This provides evidence that longer side chains on PEO surfaces inhibit FBGC formation.

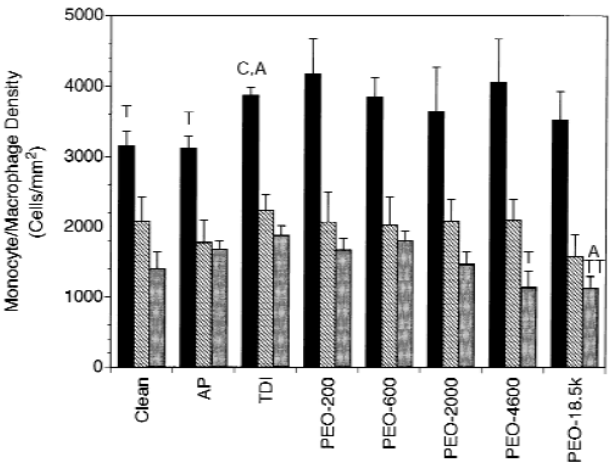

Figure 8: monocyte/macrophage density (cells/mm^2) on day 0 (black) day 3 (striped), and day 10 (gray) of monocyte culturing treated with IL-4 to induce FBGC formation. On clean glass substrate, aminopropyl modified glass (AP) substrate, 2,4-toluene diisocyanate modified glass (TDI) substrate, and polyethylene oxide modified glass (PEO) of 200, 600, 2000, 4600, and 18,500 Da. Letter code A,C,T, indicates significant difference to clean glass, AP, and TDI surfaces, respectively. With a single letter being a significance levels of $p < 0.05$. And double letters being a significance level of $p < 0.01$. Mean values and standard errors are shown, n=4 [69].

Polyetherurethane (PU)

PU is a widely used flexible biomaterial with good biocompatibility. PU is used for medical devices such as vascular prosthesis, heart valves and cardiac assist devices [70]. However, FBGC have been shown to be able to crack the surface of polyetherurethane implants after around 5 weeks [9]. In addition FBGC have been shown to be involved in both the oxidation and hydrolysis of PU [11]. The surface of the PU seems to play an important role, as it is able to modulate the formation of FBGC [11]. PU chemistry also seems to be an important factor. One study compared polycarbonate based PUs (HDI431) synthesized by hexane diisocyanate (HDI), poly(1,6-hexyl 1,2- ethyl carbonate) diol (PCN) and butanediol (BD) in a 4:3:1 ratio to a PU synthesized with 4,40-methylene-bis-phenyl diisocyanate (MDI),PCN and BD in a 3:2:1 ratio (MDI321), they found that the HDI431 lead to significantly higher amounts of FBGC than MDI321 [11].

Another study performed by Zhao et al with subcutaneously implanted in rats, not only showed differences in amount of FBGC on different PU's but also differences in degeneration of these PU's. They used four different PU's, the first was standard PU, secondly they used PU containing 1% Santowhite powder, the third contained 5% Methacrol 21386 and the fourth contained both additives. Both PU and PU containing 5% methacrol showed FBGC as early as 4 days after implantation and after 1 week these samples displayed up to 250-300 cells/cm^2. The PU samples containing Santowhite powder and both Santowhite powder and 5% methacrol displayed lower FBGC densities with a maximum of 100-150 cells/cm^2 at 2-3 weeks.

On the standard PU sample cracking of the surface started to appear after 3 weeks, in comparison the PU's containing the additives this cracking seemed lower. However on both samples containing 5% methacrol severe pitting of the sample appeared after as early as 2 weeks. This cracking of the surface was believed to be induced by oxidative degeneration, caused by oxygen radical release of FBGC showing their degenerative function. 5% methacrol is believed to cause pitting in acidic environments. It is known that FBGC can cause a highly acidic zone (pH 3.5) when attached to the surface of the biomaterial, this acidic environment caused by the FBGC may have caused the pitting of the 5% methacrol samples [71].

Candidate substances to inhibit FBGC formation on PU include IL-4 neutralizing antibody (IL4Ab), which has shown an inhibition of FBGC formation on subcutaneous implanted PU in mice [72]

Secretion of cytokines by FBGC

So far it remains relatively unclear whether FBGC are capable of the secretion of cytokines and whether or not FBGC have a role in the inflammatory response during the FBR. However it is known that in giant cell arteritis other multinucleated giant cells promote neoangiogensis through the production of angiogenic cytokines such as vascular endothelial growth factor (VEGF) [73]. This suggest that FBGC are able to secrete cytokines. It has also been suggested that FBGC are capable of producing cytokines, such as connective tissue growth factor that would induce a fibrogenic phenotype in wound healing [65].

FBGC gene expression plasticity

Luttikhuizen et al performed a study in which they compared gene expression of FBGC on different substrates including, polyethylene terephthalate (Dacron), cross-linked sheep collagen (HDSC), and bioactive PCLdiUPy [74].

Comparing the gene expression of FBGC on these different substrates reveal distinct differences (Fig. 9). PCLdiUPy displays higher gene expression for IL-1β, IL-6, CCL2, VEGF and FGF compared to the other substrates (Fig. 9). HDSC displays lower gene expression of TNFα, CXCL1, CXCL2 and CCL2 when compared to the other substrates (Fig. 9) [74]. These results indicate that FBGC are capable of secretion cytokines, chemokines, ECM, and angiogenic mediators, suggesting a role for FBGC in inflammatory and agionegenic processes. And it also indicates that the gene expression of FBGC differs between different substrates that they encounter.

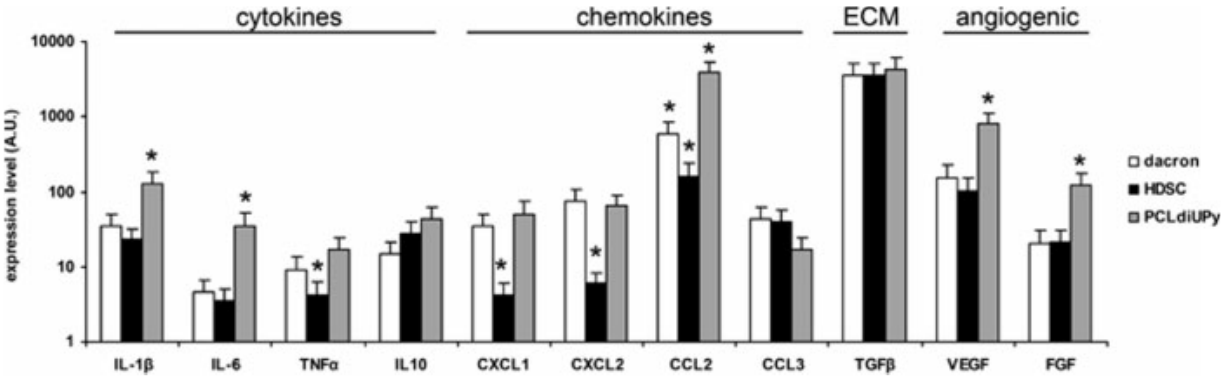

*Figure 9: gene expression of cytokines, chemokines, ECM, and angiogenic mediators by giant cells on Dacron, HDSC and PCLdiUPy substrates. Implants were explanted after giant cells had formed, this was after 21 days for Dacron and HDSC and after 10 days for the bioactive PCLdiUPy. Gene expression was measured using quantitative PCR, mean values ± SEM are shown, (*p < 0.05)* [74] .

Role of FBGC in inflammatory response

Definite proof of cytokine secretion by FBGC is provided by Hernandez-Pando et al. They observed FBGC, consisting of up to 3 nuclei, 7 days after injection with nitrocellulose in mice footpads (Fig. 10). These FBGC already stained positively for IL-1α and TNF-α (Fig. 10). Three and four weeks after injection FBGC had grown in size and IL-1α and TNF-α expression had increased. At 45 and 60 days after injection expression patterns of FBGC changed, as they then displayed strong expression of TGF-β and no expression of IL-1α and TNF-α [75].

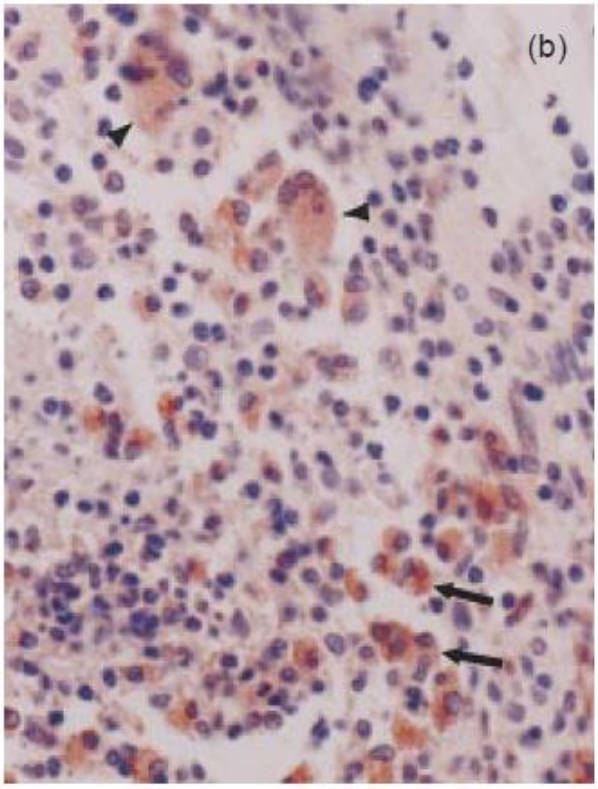

Figure 10: Macrophages (arrows) and FBGC (arrowheads) stain positively for interleukin-1α (IL-1α) 7 days after subcutaneous injection of nitrocellulose microscopic particles in mice footpads [75].

These results do not only prove that FBGC are capable of the secretion of cytokines but also indicate an active role of FBGC in the inflammatory response during the FBR.

FBGC and Diabetes Mellitus

Another important study by Socarrás et al, observed differences in diabetic versus nondiabetic rats in fibrotic capsule formation and FBGC formation when subcutaneously implanted with polyether-polyurethane sponge discs (Fig. 11). After 10 days of implantation the fibrotic capsule of nondiabetic rats had a thickness of 294.5±18.5 µm, whereas the thickness of the fibrotic capsule in diabetic animals was 169.4±10.8 µm (Fig. 11C).

So diabetic rats show significantly lower fibrotic capsule formation when compared to nondiabetic rats (Fig. 11A-C). The same result was found for FBGC formation, diabetic rats show significantly lower FBGC formation when compared to nondiabetic rats (Fig. 11D-F) [76].

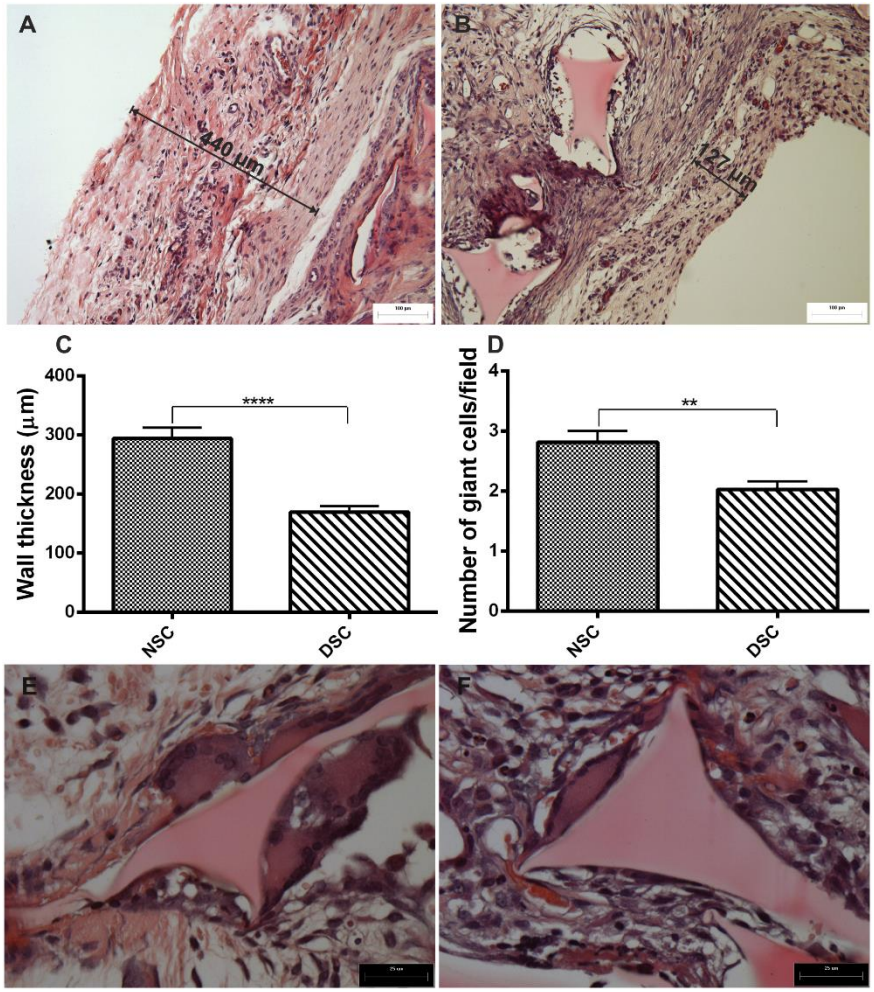

*Figure 11: Histological sections of fibrous capsule in 10-day old implants from non-diabetic (A) and diabetic rats (B) (H&E staining) scale bar = 100µm. Wall thickness was decreased in diabetic induced rats (C), mean values ± SEM are shown. Furthermore, the number foreign body giant cells was equally reduced in implants from diabetic rats (D). Values shown are expressed as mean±SEM. *Significant difference between non-diabetic and diabetic; p<0.05. Histological sections of foreign body giant cells in 10-day old implants from non-diabetic (E) and diabetic rats (F) (H&E staining) scale bar = 25µm* [76].

Polydimethylsiloxane (PDMS)

PDMS is a widely used biomaterial which is incorporated in biomedical devices such as stents. Its elastomeric properties, biocompatibility, gas permeability, optical transparency, and relatively low costs make it an attractive material [77, 78].

When PDMS was compared to low density polyethylene (LDPE), PDMS was found to display a higher activation of complement factors and more adhesion of macrophages resulting in higher FBGC formation [21]. After 3 weeks the PDMS contained more FBGC with more than 20 nuclei in comparison to the LDPE. Also the phagocytic abilities of the FBGC on PDMS were lower than on LDPE [79]. PDMS surfaces also displayed significantly higher macrophage fusion than Bionate 80A (PCU), Elasthane 80A (PEU) and PurSil20 80A (PEU-S) surfaces after 7 days and after 10 days of culturing [80]. However PDMS and other hydrogels have been found to display a lower density of FBGC, as well as a lower coverage of the surface with FBGC than Pellethane® a thermoplastic PU [81]. So tremendous differences can be found of FBGC formation, density and coverage, between different substrates.

Xenograft

Furthermore FBGC reactions have been found in patients with lower eyelid retractions using tarSys™ xenograft [82].

Ceramics

Macrophage multinucleation was investigated on biphasic calcium phosphate (BCP) ceramic in rabbits. Also defects in the BCP were created and filled with ceramic paste, referred to as dCOMP. Multinucleated macrophages appeared after 4 weeks on the ceramic material and especially in the defects of the dCOMP (Fig. 12) [83].

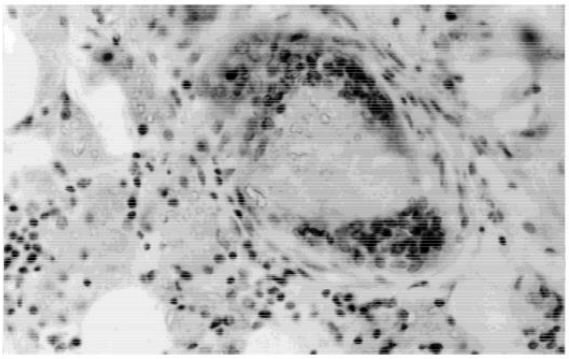

Figure 12: multinucleated giant cell in dCOMP, 4 weeks after injection into rabbit leg, 400X magnification, Hematoxylin–eosin staining [83].

Polymer networks

Collier et al compared N-(2 aminoethyl)-3 aminopropyltrimethoxysilane (EDS) and an interpenetrating polymer network (IPN) of polyacrylamide and poly(ethylene glycol) to each other. They found that FBGC formation on IPN did not occur whereas on EDS it did occur (Fig. 13). On EDS adherent macrophages displayed 46% fusion on day 7 and 40% fusion on day 10 (Fig. 13). The IPN surface however, lead to the formation of monocyte aggregates instead of FBGC formation (Fig. 13). Therefore the IPN surface prevented FBGC formation by inhibiting the differentiation of monocytes into macrophages. It has been suggested that the IPN surfaces have less protein absorption which inhibits monocyte differentiation into macrophages, this could explain why ultimately FBGC formation is inhibited [84].

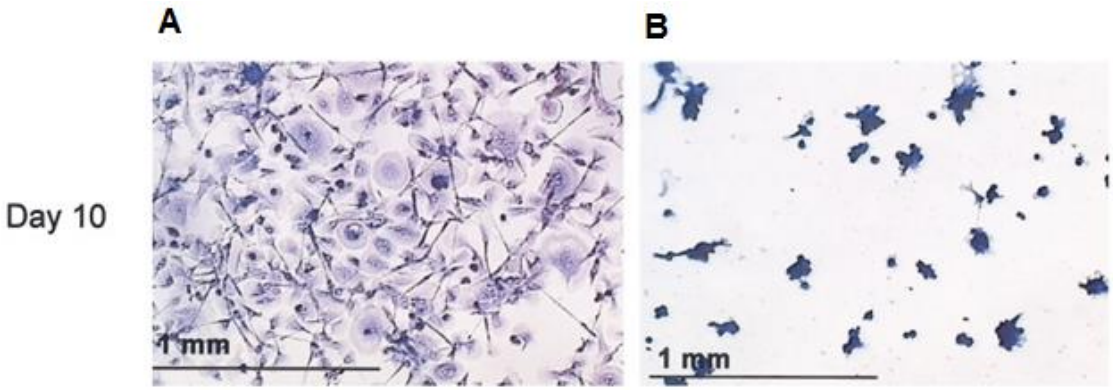

Figure 13: FBGC formation on EDS (A) and monocyte aggregation on IPN (B) after 10 days of culturing, staining with May Grunwald and Giemsa [84].

Possible targets of FBGC

This review results in several potential targets of FBGC that could prove to be clinically relevant. The first study that will be discussed is by Sterling et al, and it states that CD44 ligands, hyaluronic acid (HA), chondroitin sulphates (CSA), and osteopontin (OP) prevent macrophage fusion. CD44 has been reported to be involved in macrophage fusion as previously discussed. Now, CD44 ligands HA, CSA and OP have shown to prevent macrophage fusion in a dose dependent way (Fig. 14). This effect was not only found for the soluble ligands but also for plastic surfaces coated with these ligands [56].

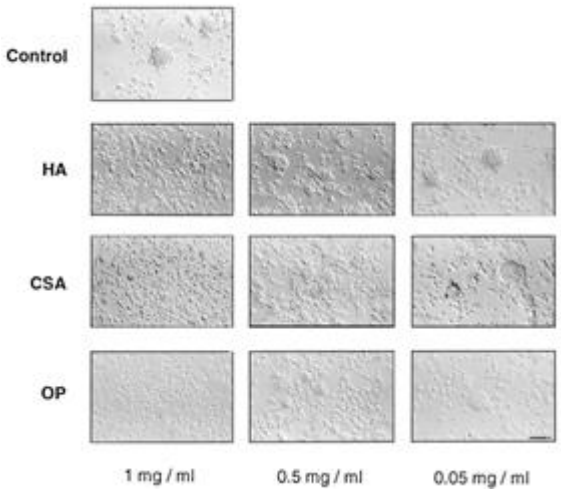

Figure 14: dose dependent inhibition of macrophage fusion by hyaluronic acid (HA), chondroitin sulfates (CSA), and osteopontin (OP) on a plastic surface [56].

Several possibilities were discussed as to how these ligands inhibit macrophage fusion. One possibility is that they activate macrophages, which prevents fusion. Another possibility is that they compete with the cell surface ligands of other macrophages preventing cell-cell adhesion and thereby fusion [56]. The last, and probably most interesting possibility, is that these ligands result in a redistribution of the CD44 receptors on the membrane of the macrophage. Previously the polarisation of Na-K-ATPases to the non-adhesive side of the macrophage membrane has been discussed. For the CD44 receptors polarisation to the adhesive side of the macrophage membrane as a result of the CD 44 ligands has been proposed. This could mechanical prevent adhesion molecules from reaching the CD 44 receptors, which in turn inhibits macrophage fusion [56]. However the precise mechanism by which CD44 ligands prevent macrophages from fusing still remains unclear. But knowing that these CD44 ligands prevent macrophage fusion could prove to be clinically relevant in inhibiting FBGC in the foreign body response.

The second discussed is by McNally, which reports the involvement of antioxidant vitamin E (90% α-tocopherol), by activation of diacylglycerol
Kinase, in macrophage fusion [46]. Persistent with this finding the diacylglycerol kinase inhibitor R59022 was found to prevent FBGC formation [46]. This effect again proves to be dose dependent, showing almost complete inhibition of FBGC formation with a concentration of 15 µM R59022 (Fig. 15) [46]. However macrophage adhesion does not seem to be affected by R59022.

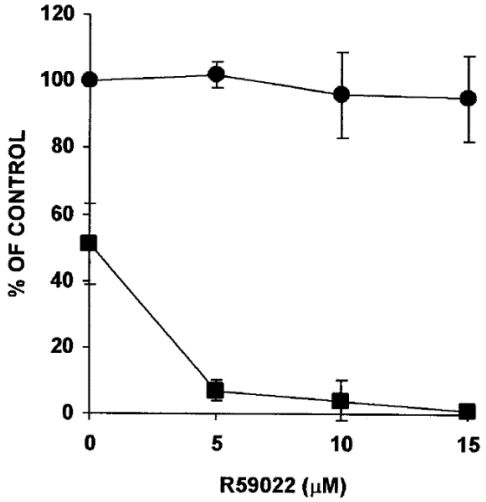

Figure 15: dose dependent inhibition of macrophage fusion by diacylglycerol kinase inhibitor R59022 on IL-4-treated cultures. The indicated concentrations of R59022 were added to day 3 macrophages, which were stained on day 7 with May-Grünwald/Giemsa. The results are indicated as a percentage of macrophage adhesion (circles) or a percentage of FBGC formation (squares) ± SEM, n= 3 monocyte donors [46].

These results could indicate a prominent role for phosphorylation of diacylglycerol in FBGC formation. The R59022 inhibitor could therefore prove to be clinically relevant in preventing macrophage fusion.

The third study that presents a candidate for inhibition of macrophage fusion is the study performed by Zhao et al on PU coatings, where they found that Santowhite powder coatings resulted in inhibition of FBGC formation [71]. In addition the studies by Jenny et al, also proposed inhibitory effects of coating. They found that tetradecyl (C14) and octadecyl (C18) alkyl coatings on glass resulted in inhibition of FBGC formation [68]. As well as glass coated with 18.500 Da PEO [69]. These results indicate that different coatings of biomaterials could definitely have an effect on the foreign body response against these biomaterials, especially the formation of FBGC on the surface of these biomaterials.

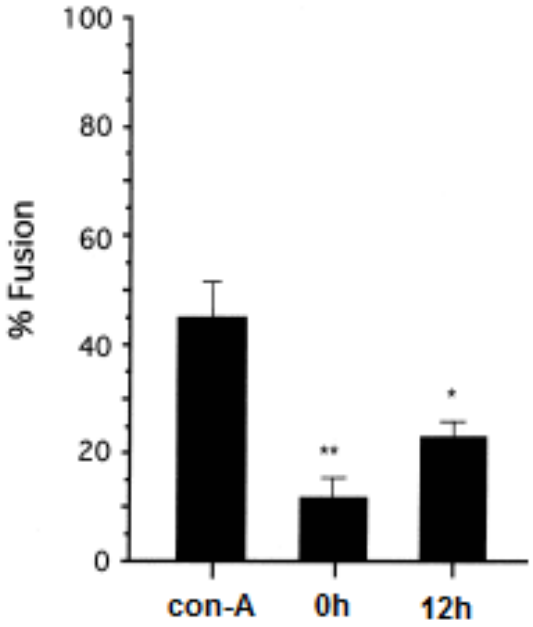

*Figure 16: Macrophage fusion of human monocyte culture in 10 % FCS RPMI medium containing 10 µg/ml of Con A. 1 mM ferric citrate was added after 0h and 12 hours. Each bar represents the mean ± SEM of 3 experiments. * $p < 0.05$, ** $p < 0.02$* [50].

The fourth study that will be discussed is by Tahara et al, where the researchers found that macrophage fusion by con A can be inhibited by addition of 1mM ferric citrate (Fig. 16). However this effect is only short term as the effect is significant directly after addition of ferric citrate up to 12 hours after addition (Fig. 16). After 24 hours the effect is not significant anymore [50]. This reveals a short term effect of ferric citrate on FBGC formation. Because this effect is only short term, the clinical applications would be limited.

The fifth study by Sorimachi et al shows the effect of anti-TNF-α on the fusion of macrophages derived from rat bone marrow (Fig. 17). Anti-TNF-α inhibits macrophage fusion of rat bone marrow derived macrophages (Fig. 17) [85]. However since to my knowledge no studies were performed on human macrophages this effect has little clinical value.

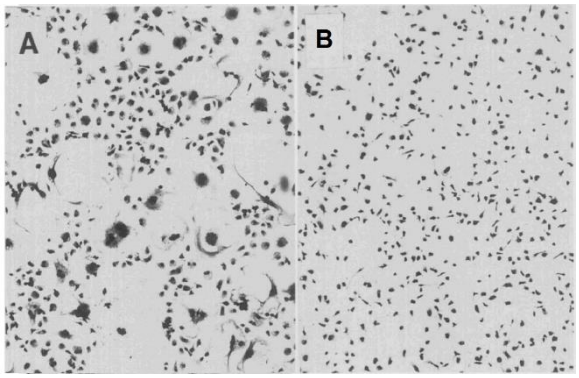

Figure 17: Effect of monoclonal antibody for recombinant human TNF-α (10µl/ml in medium) ***(B)*** *or medium containing 10µg/ml acetyl lignin (EP3)* ***(A)*** *macrophage fusion. Macrophages were derived from rat bone marrow. Samples were cultured for 3 days, fixed with methanol and stained with Giemsa staining* [85].

The sixth study that will be discussed that poses a candidate for inhibition of macrophage fusion is a study performed by Katsuyama et al. They show that interleukin-1 receptor-associated kinase-4 (IRAK4) inhibits FBGC formation [47]. IRAK4 is arguably the best potential candidate for clinical use. Since it has been shown that IRAK4 deficient mice display normal osteoclast formation and bone mineral density [47]. In comparison IRAK4 was found to inhibit macrophage fusion [47]. In addition IRAK4 deficiency restored macrophage fusion [47]. Normal osteoclast function in combination with FBGC formation inhibition classifies IRAK4 as a potential therapeutic target for inhibition of macrophage fusion during the FBR.

In the last study that will be discussed, Tsai et al show osteopontin (OPN) as another possible candidate for inhibition of FBGC formation, both in vitro and in vivo. OPN knock out mice displayed an increase in FBGC formation. And in vitro results show a dose dependent inhibition of FBGC formation by OPN (Fig. 18). However a relatively high dose of OPN is required for prevention of fusion (Fig. 18) [86].

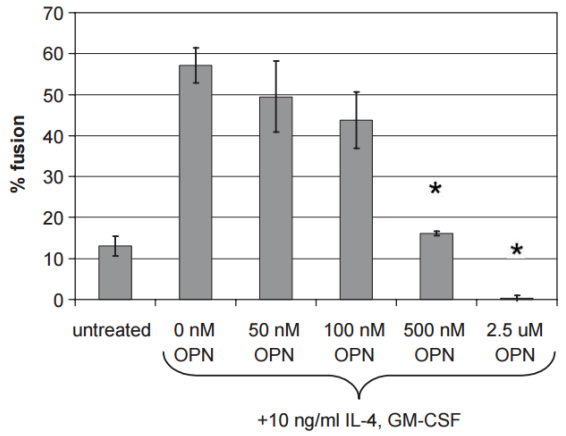

*Figure 18: macrophage fusion in vitro. Fusion percentage is the number of nuclei within FBGC (more than 3 nuclei)/total number of counted nuclei *100%. * is significant at p=0.0001. N=3 wells per sample, mean values and SD are shown* [86].

FBGC versus mononuclear macrophages

Earlier the differences in the Ym1 and ALOX15 markers between FBGC and M2 macrophages were discussed, stating that Ym1 and ALOX15 expression is significantly higher in FBGC than in M2 mononuclear macrophages [39]. However there are more differences between FBGC and mononuclear macrophages that have been reported.

Mononuclear macrophages in vitro possess higher locomotive ability than multinucleated macrophages. As far as phagocytosis activity goes, mononuclear macrophages display a higher rate of phagocytosis of smaller particles whereas giant cells are typically found to surround larger particles, indication differences in phagocytosis function [30].

Other studies support the finding that mononuclear macrophages display phagocytosis of biomaterials, whereas this is not found for FBGC. Multiple reports of intracellular biomaterial particles in mononuclear macrophages support their phagocytic abilities towards biomaterials [83, 87]. Intracellular biomaterial particles have however not been reported in FBGC, questioning their phagocytic abilities.

In a comparative study between mononuclear and multinuclear macrophages performed by Papadimitriou et al, it was found that multinucleated macrophages have significantly higher protein content than mononuclear macrophages. However no significant differences in succinate dehydrogenase activity, non-specific esterase activity or acid phosphatase activity were found between mononuclear macrophages and giant cells. All values were compared per nuclei in order to equally compare mononuclear and multinuclear macrophages [88].

The most striking result is that succinate dehydrogenase activity, non-specific esterase activity and acid phosphatase activity of FGBC were found to be equal to those of mononuclear macrophages. This excludes the role of FBGC in the FBR as being more efficient in breaking down biomaterials. However the increased functionality of FBGC with regard to these degrading enzymes might not so much be limited to the amount of secreted enzyme, but could very well be in the focussed delivery of these enzymes by FBGC. Since FBGC might be able to deliver these enzymes

on a smaller surface area than multiple mononuclear macrophages could. This is yet another example that the functionality of FBGC in the FBR remains unclear.

Another comparative study between mononuclear and multinuclear macrophages performed by Enelow et al, revealed different results. They measured oxidative activity with two different techniques. First oxidative activity was measured by cytochrome-c reduction measurements, this revealed a 2.2 fold increase in oxidative activity of multinucleated giant cells as appose to mononuclear macrophages. With giant cells producing 34.3 ± 8.4 nmol of superoxide/µg of cellular protein and mononuclear macrophages producing 16.2 ± 4.5 nmol of superoxide/µg of cellular protein. Secondly, fluorescence intensity revealed that giant cells had 1.7 times brighter fluorescence per unit of cytoplasm compared to mononuclear macrophages. These results indicate that giant cells have an increased superoxide anion production compared to mononuclear macrophages [89].

In another study however, it has been shown that FBGC are capable of an 20-30 fold higher secretion of oxygen free radicals per cell in comparison to normal macrophages in response to zymosan [90]. So notable differences in oxygen free radical production by FBGC have been reported in the literature.

Enelow et al, not only investigated the superoxide anion production, but also compared the phagocytosis and killing of Candidae albicans (a gastrointestinal yeast) of mononuclear macrophages and giant cells. They found no significant differences in the number of Candidae phagocytized nor in the number of cells displaying phagocytic activity [89]. However they did find significant differences in killing of Candidae between macrophages and giant cells. As giant cells killed 35.1% ± 2.0% of phagocytized organisms, whereas macrophages killed 22.9% ± 1.8% of phagocytized organisms. This experiment reveals the phagocytic activity of multinucleated giant cells, as well as their ability to kill phagocytized organisms (Fig. 19) [89].

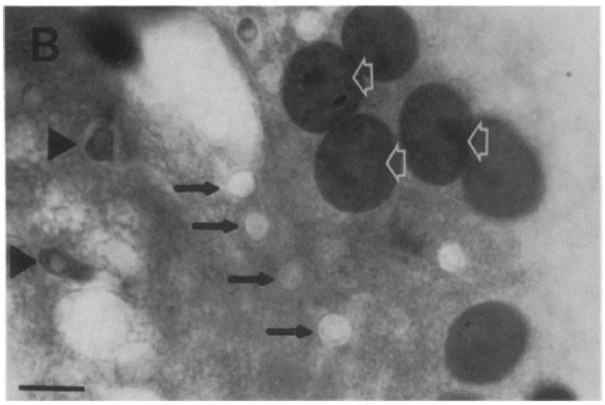

Figure 19: Multinucleated giant cell phagocytizing Candidae. Giant cell nuclei are indicated with open arrows, candidal "ghosts" are indicated with arrows and candidae surviving at time of fixation are indicated with arrowheads. Bars = 10 um [89].

However controversial results have been found regarding the phagocytosis of multinucleated giant cells. As Papadimitriou et al observed an decrease in both yeast and staphylococci ingestion by macrophages as they fuse (Fig. 20). As the number of nuclei increases the phagocytic ability decreases (Fig. 20) [91]. So whereas Enelow et al, found that multinucleated giant cells phagocytize Candidae, Papadimitriou et al found that phagocytic ability degreases with the amount of nuclei.

No. of nuclei within cells	No. of yeasts ingested/cell	No. of staphylococci ingested/cell
1	7.7 ± 3.2	59.8 ± 20.5
2	7.3 ± 3.4	61.4 ± 12.6
3	6.7 ± 2.8	51.2 ± 12.1
4	6.6 ± 2.2	46.1 ± 16.5
5	6.9 ± 2.4	47.3 ± 14.3
6	6.7 ± 2.7	48.3 ± 13.4
7+	0.04 ± 0.32	3.2 ± 1.2

Figure 20: progressive decrease in phagocytosis of yeast and staphylococci with increase in number of nuclei of giant cells [91].

Conclusion

To summarize, FBGC are believed to cause oxidation and hydrolysis of biomaterials. This is supported by the fact that FBGC showed to release up to 30 times more oxygen free radicals than mononuclear macrophages. They are also believed to be able to secrete cytokines chemokines, ECM, and angiogenic mediators.
Furthermore there have been differences found in FBGC formation between different species of animals, different biomaterials, different compositions of the same biomaterial, different surfaces of the biomaterial, as well as different sites of implantation of the biomaterial.
Controversial results were found in the literature with regard to phagocytic ability and the secretion of degeneration enzymes of giant cells in comparison to mononuclear macrophages. However in gerenal mononuclear macrophages seem to display phagocytosis whereas FBGC typically surround the biomaterial.

Future research

This review concludes that controversial results were found in the literature with regard to FBGC. This implies that further research should be conducted. In this part of the review, future prospects for research on FBGC will be discussed.

The first option that will be discussed builds on the results of Jenny et al, they investigated the effect of silane modified glass with alkyl and PEO side chains on FBGC formation [68, 69]. They found that longer side chains inhibited FBGC formation, either through inhibition of macrophage adhesion or direct inhibition of FBGC formation. Also different surface textures were found to influence FBGC formation. However the a combination of these experiments has not yet been performed. Surfaces modified with cone like shapes of different lengths could prove the effect of both surface texture and side chain length on FBGC formation. It is known that cone like shaped surfaces effect cell attachment of fibroblasts [92]. Cone shape modified surfaces might also prove to be effective in the inhibition of FBGC formation. The spacing of the cones can be modified as well [92]. This could simulate a more smooth surface instead of textured, which was found to inhibit macrophage adhesion [66]. By providing side chains to the surface by means of modifying the surface with cone shapes, and applying enough spacing between the cones, FBGC formation might be inhibited. As this would combine the effects of side chains and limit the macrophage adhesion of a textured surface. So testing these effects might prove to be a significant contribution to our knowledge about FBGC formation.

The second option for further research builds on the effect of diabetes on FBGC formation found by Socarrás et al. They showed that diabetic rats displayed inhibition of FBGC formation [76]. This effect might be due to the increase in blood glucose in diabetes. This increase in blood glucose could in turn affect the protein absorption to biomaterial surfaces. Which could in turn affect FBGC formation. Whether this inhibition of FBGC formation in diabetes is due to the increase in blood glucose could be investigated in a simple way by testing FBGC formation on glucose coated biomaterials. If FBGC formation on glucose coated biomaterials is lower, than this could provide evidence for FBGC formation inhibition due to differences in protein absorption.

The third option for further experiments would be on FBGC phagocytosis. Even though many articles claim phagocytic ability of FBGC, extensive search through the literature provided only one example which could prove phagocytosis by FBGC [89]. Which is surprising since experimentally phagocytosis is simple to prove. If one adds latex beads to IL4 stimulated macrophages, phagocytosis should be simple to prove using confocal microscopy. If beads are found within FBGC in such an experiment, it would provide evidence that FBGC are capable of phagocytosis.

The last option for further research is one following my personal curiosity to this subject, which is FBGC formation in HIV patients. Macrophages can be infected by HIV, multinucleated giant cells are also believed to be vulnerable to HIV infection [93]. Macrophages from early HIV patients were found to be able to fuse and form multinucleated giant cells [94]. However giant cell formation in advanced HIV patients is more rare [94]. It would be interesting to investigate the FBGC formation of HIV patients, and their FBR to implants. As their FBR might be less severe due to the HIV infection of macrophages.

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