J Mater Sci: Mater Med (2010) 21:1665–1676
DOI 10.1007/s10856-010-4015-7

Role of immune cells and inflammatory cytokines in regulation of fibrosis around silicone expander implants

Josna Joseph · Mira Mohanty · P. V. Mohanan

Received: 17 October 2009 / Accepted: 28 January 2010 / Published online: 17 February 2010

Abstract This study aimed to investigate the progress of wound healing around silicone expander with particular emphasis on fibroblasts, myofibroblasts and collagen in the repair phase. Semi-quantitative evaluation of inflammatory cells and their cytokines, fibroblasts and myofibroblasts at the tissue–material interface was carried out. Commercially available silicone expander was implanted in gluteus muscle of young female Wistar rats for 3, 7, 14, 30, 90 and 180 days. Ultra high molecular weight polyethylene served as control. The cellular response was studied by immunohistochemistry and Transmission Electron Microscopy. A thick collagenous fibrous capsule was observed around the silicone expander at 180 days, with persistent myofibroblasts, lymphocytes and macrophages as compared to the thin fibrous encapsulation around the UHMWPE implants. The regulatory role of cytokines and immune cells in myofibroblast persistence in tissue–implant interface around silicone expander has been extensively studied. Results of this study indicate the need to elucidate the signaling molecules in the transition of fibroblast to myofibroblast around silicone expander implants.

J. Joseph · M. Mohanty (✉)
Division of Implant Biology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Satelmond Palace, Poojapura, Trivandrum, Kerala 695012, India
e-mail: miramohanty@yahoo.com

P. V. Mohanan
Division of Toxicology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Satelmond Palace, Poojapura, Trivandrum, Kerala 695012, India

1 Introduction

Reports of adverse reactions to long-term implants like silicone breast implants are of increasing concern [1]. After their ban in 1992 by FDA due to multiple local complications, silicone implants were reintroduced into the market in 1999 with preference to saline filled implants. One of the major complications associated with silicone implants is capsular contracture, or the tightening of scar tissue around implant, mal position and rupture or leakage of the implant. Apart from silicone gel implants, saline implants are also not free from risks. Silica has been reported in lymph nodes in saline implant patients, implicating the migration of silicone from the implant [2].

Examination of tissue around retrieved silicone breast implants have revealed either a severe chronic inflammation or an increased amount of fibrosis [3]. The tissue response to a biocompatible material follows a well defined course of events to the injury itself. Tissue destruction is followed by acute inflammation, formation of granulation tissue and finally repair in the form of collagen deposition. The extent of this response depends on various material characteristics as well as presence of various cytokines, growth factors and proteins [4]. Variable number of inflammatory cells have been noted in the peri-implant fibrous tissue around silicone implants [5].

The termination of acute events with the formation of a fibrous capsule is the result of the delicate balance between pro- and anti-fibrotic cytokines. These include pro-fibrotic cytokines, Transforming growth factor beta (TGFβ), Connective tissue growth factor (CTGF) and anti-fibrotic ones like Tumor Necrosis factor alpha (TNFα) and Interferon gamma (IFNγ). The observation of increased fibrosis around the materials following long-term residence in tissues with a concomitant presence of inflammatory cells

leads one to speculate on the occurrence of a tilt in the balance of cytokines produced, namely either an increase in pro-fibrotic cytokines or a decrease in anti-fibrotic cytokines.

Laitung et al. have suggested long-term animal studies with tissue expanding devices to elucidate the early and late phases of scar formation [6]. Experimental studies in rats have dealt with study of the tissue response around silicone elastomer. However, these studies have been short termed and are related to the tumorogenic potential of this material [7].

This study was undertaken to examine in vivo, the progress of wound healing around silicone expander (SE) with particular emphasis on fibroblasts, myofibroblasts and collagen in the repair phase. We report the results of a semi-quantitative evaluation of inflammatory cells and their cytokines, fibroblasts and myofibroblasts at the tissue–material interface. The significance of this study is that the soft-tissue reaction around the SE in comparison to ultra high molecular weight poly ethylene (UHMWPE) has been analyzed at regular intervals over a period of 180 days.

2 Materials and methods

2.1 Materials

SE which is commercially used for breast augmentation was selected for the study. UHMWPE, a known reference material cited by ISO 10993:12 [8] for evaluation of biocompatibility was used as experimental control. Both materials were cut into pieces of 1.5 × 1.5 × 1 cm, cleaned and sterilized with ethylene oxide.

2.2 Implantation and Retrieval

Sterile pieces of SE and UHMWPE were implanted in gluteus muscle of young female Wistar rats. Animals were categorized into 6 groups of 8 animals each. Animal experiments were approved by Institute Animal Ethics Committee. The implantation procedure was carried out based on ISO-10993-6 [9]. Implantation was done aseptically after anesthetizing the animals by administering 80 mg ketamine + 5 mg xylaxine/kg body weight intramuscularly. The skin was swabbed with 70% alcohol and a 1.5 cm incision was made in the gluteus muscle. The SE material of 1.5 × 1.5 cm was inserted into the muscle and the wound was closed with catgut and the skin sutured externally with a nylon suture. UHMWPE material was implanted in the contra lateral leg. Post-implantation care was given according to the guidelines of the Institutional Animal Ethics Committee and the animals were provided with food and water ad libitum. At the end of the study period, animals were sacrificed with an overdose administration of sodium thiopentone. Implants with surrounding tissue from each group were retrieved at 3, 7, 14, 30, 90 and 180 days post-implantation. The implants were carefully removed from the peri-implant tissue and fixed in phosphate buffered saline (PBS) for Fourier Transform Infrared (FTIR) spectroscopy analysis. The retrieved tissues were partly fixed in (i) 10% buffered formalin for studying collagen deposition by Trichrome staining, (ii) partly in 3% buffered gluteraldehyde for transmission electron microscopy (TEM) analysis and (iii) partly snap frozen in liquid nitrogen cooled isopentane for immunohistochemical analysis.

2.3 FTIR spectroscopy

Molecular structure of SE pre- and 6 months post-implantation was studied by a NICOLET 5700 FTIR in an ATR mode. The spectral pattern obtained was matched with known materials in Hummel polymer sample library using OMNIC-2 software. Change in spectral pattern was looked for in both samples.

2.4 Collagen deposition

The formalin fixed tissues were dehydrated in ascending grades of alcohol, cleared in chloroform and embedded in paraffin wax. Collagen deposition in the tissue around the implant was studied by Masson's Trichrome staining in 5 μm thick paraffin sections. The sections were deparaffinized in xylene and hydrated in ascending grades of alcohol to deionised water. They were then fixed in preheated bouin's solution at 56°C for 15 min. Washed in running tap water and stained with Harris' Heamatoxylin for 5 min. Washed in water and stained with Masson's Trichrome stain (Trichrome stain LG solution, HT10316, Sigma–Aldrich, USA) for 5 min. The sections were differentiated in 0.5% glacial acetic acid for 1 min. Rinsed the slides and dehydrated through alcohol, cleared in xylene and mounted with cytoseal 60 (# 18006, Electron Microscopy Sciences, PA). The stained sections were observed for collagen deposition by bright field microscopy (Nikon Eclipse E 600, Japan).

2.5 Identification of immune cells and pro-inflammatory cytokines in peri-implant tissue

Immuno phenotyping of specific cells and their cytokines was carried out using immunohistochemistry. On 8 μm thick cryo sections of liquid nitrogen fixed peri-implant tissue obtained with a cryomicrotome (Leica CM 3050 S, Germany), immunostaining was performed using the

Ultratech HRP Streptavidin–Biotin detection system (PNIM2765, Beckman Coulter, USA). Briefly, the cryosections were fixed in cold acetone at −20°C for 10 min, followed by rehydration in PBS for 10 min, and blocked with Hydrogen peroxide (H_2O_2) and protein blocking agent for 10 min each. The excess liquid was wiped off from the sections and incubated with primary antibody in appropriate dilution (Table 1) for 1 h in humidity chamber, washed in PBS and subsequently incubated with biotinylated secondary antibody for 20 min. The sections were then incubated with streptavidin-peroxidase reagent for 20 min and peroxidase substrate and chromogen solution, 3,3′-diaminobenzidine (DAB) for 5 min. Counterstaining was done with Harris Heamatoxylin, sectrions air dried and mounted with mounting medium and evaluated using a bright field microscope.

The Immunopositive cells were qualitatively graded as few (+) and scattered; mild (++) with small collections of cells; moderate (+++) and numerous (++++) cells at tissue–implant interface. The cytokines were graded qualitatively depending on intensity of staining as mild (+): moderate (++) and intense (+++).

2.6 Transmission electron microscopy

Gluteraldehyde fixed tissues were cut to pieces that include tissue–implant interface. The tissues were washed with cold phosphate buffer (pH 7.4), 4 changes, for 10 min each, with sample vials standing in an ice bath. Tissues were placed in 1% OsO_4 for 2 h and thereafter washed with phosphate buffer, 4 changes for 15 min each, in an ice bath, rinsed in distilled water for 5 min, followed by dehydration in ascending grades of acetone, infiltrated in Polybed 812 (Polyscience Inc., USA) and embedded in resin. 1 μm thick Ultrathin sections taken with an ultramicrotome (Lieca ultracut model, UCT, Germany) were stained with Uranyl acetate for 2 h. Thereafter, sections were stained with Lead citrate for 10 min, washed, air dried and observed under the electron microscope (Model Hitachi-600) at an accelerating voltage of 75 kV, photographs(Kodak Illford film) were captured.

Table 1 Primary antibodies used for the immunohistochemical analysis

Antibody	Cat. no.	Dilution
ED2 (macrophage lysosomal antigen)	MCA 342R	1:100
CD4 (T lymphocytes)	MCA 55G	1:100
Vimentin (fibroblasts)	sc-7557	1:200
Alpha smooth muscle actin, αSMA (myofibroblasts)	sc-1615	1:100
Transforming growth factorβ	sc-146	1:250
Tumor necrosis factorα	sc-1350	1:500
Interleukin 1α	sc-1254	1:500
Interleukin 1 β	MCA1397	1:250
Interferonγ	MCA1301	1:25
Interleukin-6	sc-1265	1:500
Interleukin-10	sc-1783	1:250

3 Results

3.1 FTIR spectroscopy

The spectral pattern obtained for SE pre-implantation showed peaks matching with spectrum of standard, poly dimethyl siloxane. Except for a change in the aliphatic hydrocarbon groups at 1460 cm^{-1}, the peaks in pre- and post-implantation were similar (Fig. 1).

3.2 Masson's Trichrome staining

Trichrome staining revealed deposition of collagen around both implants at 30 days following implantation. Collagen gradually increased over time with a thick capsule of collagen being present at 180 days (Fig. 2e). On the other hand scant collagen was observed the UHMWPE interface at all time periods (Fig. 2b, d, e).

3.3 Immunohistochemistry

3.3.1 Cells

Immunophenotyping of different cells present at the tissue–material interface showed an initial large number of macrophages around both implants at 3, 7, 14 and 30 days. *Macrophage* infiltration remained steadily high around silicone at 90 days with a gradual decrease to a few cells at 180 days (Fig. 3b, c).The number was slightly less around UHMWPE at 90 days and absent at 180 days whereas they were persistent around silicone even at 180 days (Fig. 4b, c). *T Helper cells* were present in large numbers around silicone at 30 days with a gradual reduction noted at 90 days and remained steady at 180 days (Fig. 3d, e, f). Cells were absent around UHMWPE at 180 days. *Fibroblasts* were few at the silicone–tissue interface at 30 days which increased at 90 days and reduced to few at 180 days (Fig. 3i). A similar trend was noted around UHMWPE at same time periods. At 30 days few *Myofibroblasts* were present around silicone but occasional around UHMWPE. An increase was noted at 90 days which remained steady at 180 days (Fig. 3k, l). On the other hand myofibroblasts were always fewer around UHMWPE at early time periods

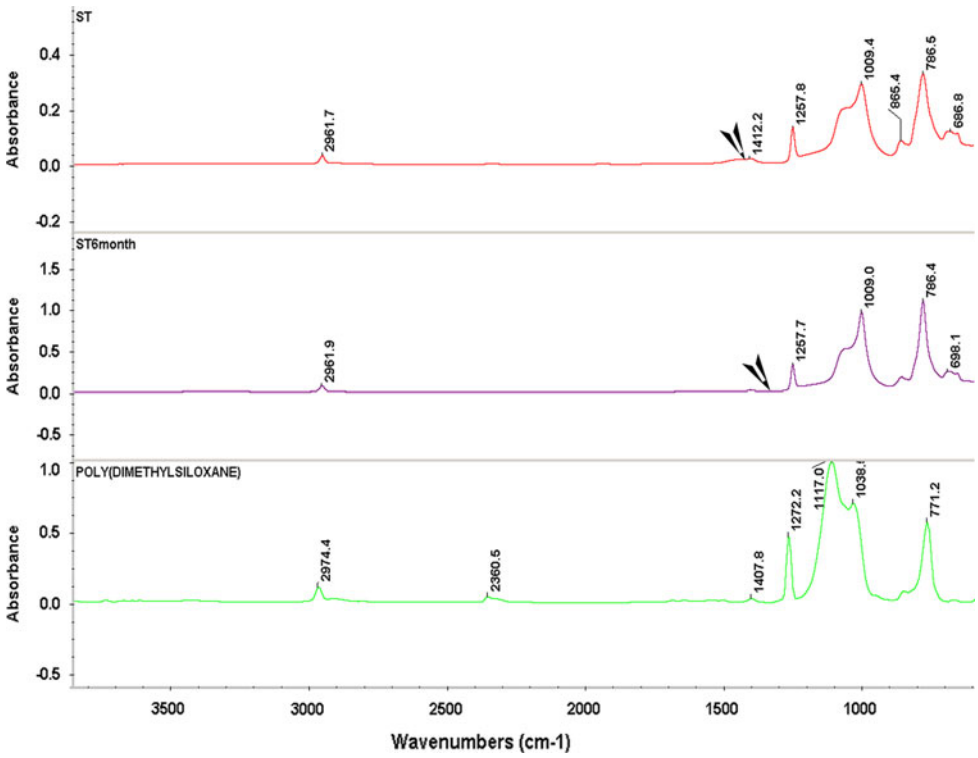

Fig. 1 FTIR Spectrum of SE material before implantation and 180 days post-implantation. **a** pre-implantation, **b** 180 days post-implantation and **c** standard spectrum of Poly Di Methyl siloxane

and absent at 180 days (Fig. 4k, l). The semi-quantitative evaluation data is summarized in Table 2.

3.3.2 Cytokines

TGFβ was intensely expressed at tissue–SE interface at 30 days (Fig. 5a) which decreased to moderate levels at 90 days (Fig. 5b) and was mild at 180 days (Fig. 5c). The cytokine was mild around UHMWPE at 90 days (Fig. 6b) and absent at 180 days (Fig. 6c). The pro-inflammatory cytokine TNFα was moderately expressed at 30 days around SE (Fig. 5d), with gradual reduction over 90 days (Fig. 5e) being present in focal areas and mild at 180 days (Fig. 5f). The cytokine was much less around UHMWPE at 90 days (Fig. 6e) and absent at 180 days (Fig. 6f). IL-1α was expressed at similar levels of TGFβ at 90 days around SE (Fig. 5h) with mild expression at 180 days (Fig. 5i). The cytokine was intensely expressed around UHMWPE at 30 days (Fig. 6g), moderately at 90 days (Fig. 6h) and was absent at 180 days (Fig. 6i). Interleukin-1beta (IL-1β) was moderately expressed around SE at 90 days (Fig. 5k) and was mild at 180 days (Fig. 5l). The expression was intense around UHMWPE at 90 days (Fig. 6k) and remained steady at moderate levels at 180 days (Fig. 6l).

The collagenase stimulating cytokine IFNγ was intensely expressed around SE at 30 days (Fig. 5m) reduced to mild at 90 days (Fig. 5n) but increased to moderate at 180 days (Fig. 5o). It was expressed moderately around UHMWPE at 90 days (Fig. 6n) but reduced to mild at 180 days (Fig. 6o). Interleukin-6 (IL-6) was moderately expressed at 90 days around SE (Fig. 7a) with expression being slightly more around UHMWPE (Fig. 7c). Expression reduced but persisted around SE at 180 days mildly (Fig. 7b) but was absent around UHMWPE (Fig. 7d). Moderate expression of Interleukin-10 (IL-10) was there around SE at 90 and 180 days with no reduction in expression (Fig. 7e, f) where as around UHMWPE it reduced from mild at 90 days (Fig. 7g) to nil at 180 days (Fig. 7h). The results are summarized in Table 3.

3.4 Transmission electron microscopy

The electron micrographs revealed the presence of large amounts of collagen arranged in parallel bundles (Fig. 8c–f). The characteristic banding pattern of collagen bundles were visible. Presence of both fibroblasts and myofibroblasts adjacent to the collagen bundles were observed around SE (Fig. 8c).While normal fibroblasts contain a well developed rough endoplasmic reticulum with dilated cisternae and oval nucleus, myofibroblasts in addition to an abundant rough endoplasmic reticulum, neo express bundles of microfilaments with dense bodies [10]. Cells with intra cytoplasmic actin bundles and fibronexuses, which are characteristics of myofibroblasts were noted at 180 days (Fig. 8b, c). The cells and the collagen bundles appeared to be arranged parallel to each other. Most of the fibroblasts were in synthetic stage with numerous large mitochondria and rough endoplasmic reticulum fibres. Numerous Rough

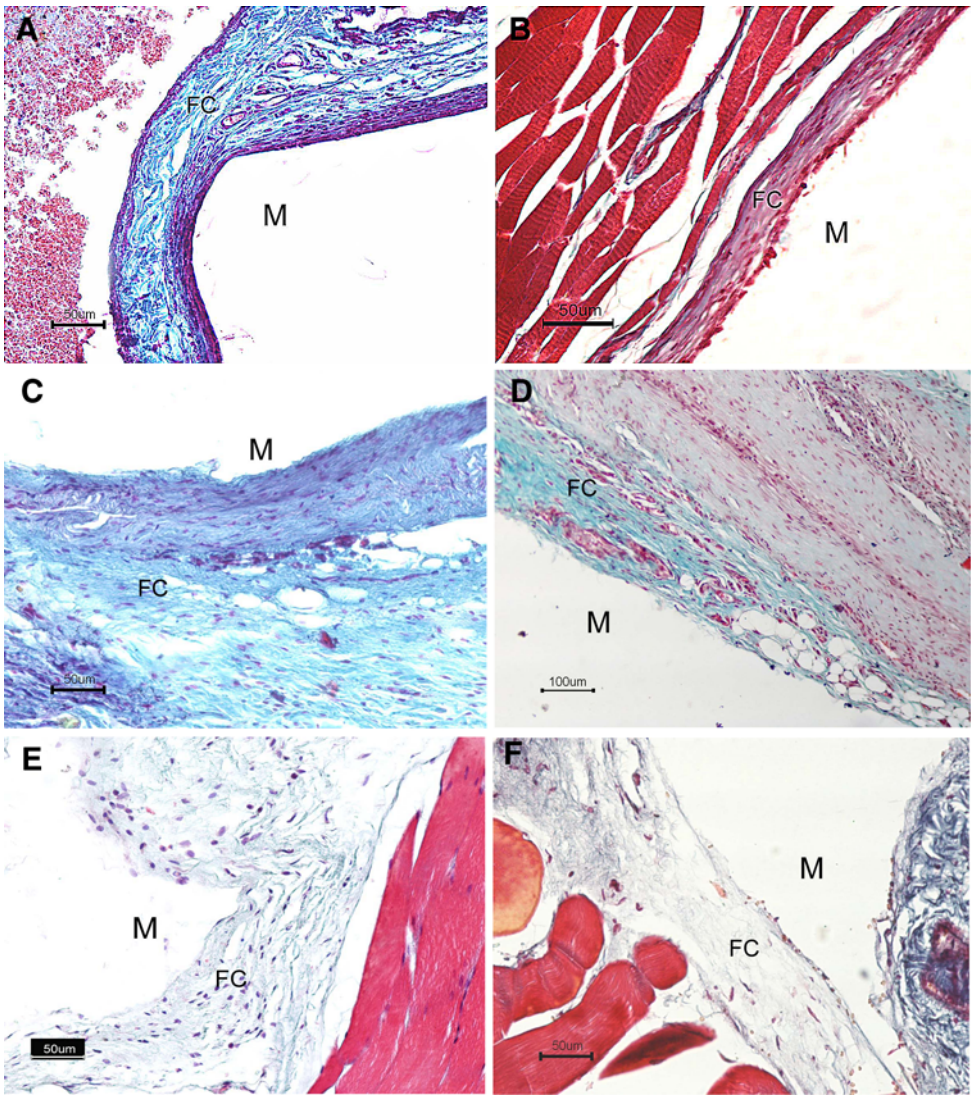

Fig. 2 Collagen deposition in peri-implant tissue around SE: 30, 90, 180 days (**a**, **c**, **e**) and UHMWPE: 30, 90, 180 days (**b**, **d**, **f**) (M, Implant site; FC, Fibrous capsule)

endoplasmic reticulum and mitochondria, indicative of active state of cells were observed (Fig. 8b).

4 Discussion

Silicone breast implants are widely used in the field of plastic surgery, for both post-mastectomy reconstruction and cosmetic augmentation. Long-term complication of silicone elastomer breast implants is capsular contracture, due to a thick fibrous capsule formation around the implant. The fate of the implant in host is determined by both the physico-chemical properties of the implant as well as the various biological components to which the implant is exposed to in the body. There was no major changes in surface moieties of SE during the wound healing phase. The FTIR analysis showed only a minor shift in peaks corresponding to aliphatic hydrocarbons before implantation and after 180 days of implantation.

In this study initial infiltration of macrophages and lymphocytes along with fibroblasts around both SE and UHMWPE is similar to that observed in the progress of healing of an incision by first intention. The difference in the healing phase around both materials is notable with the persistence of few macrophages, lymphocytes and myofibroblasts in a thick collagenous capsule around the SE at 180 days post-implantation. In contrast there is an absence of these cells in the thin fibrous capsule around UHMWPE at the same time period.

It has been reported earlier that the fibrous capsule forms around an implant usually within a short time of 2 months [11]. In our study the thickness of collagen capsule was seen to increase to maximum at 90 days and decrease gradually with persistence of a thin layer at 180 days

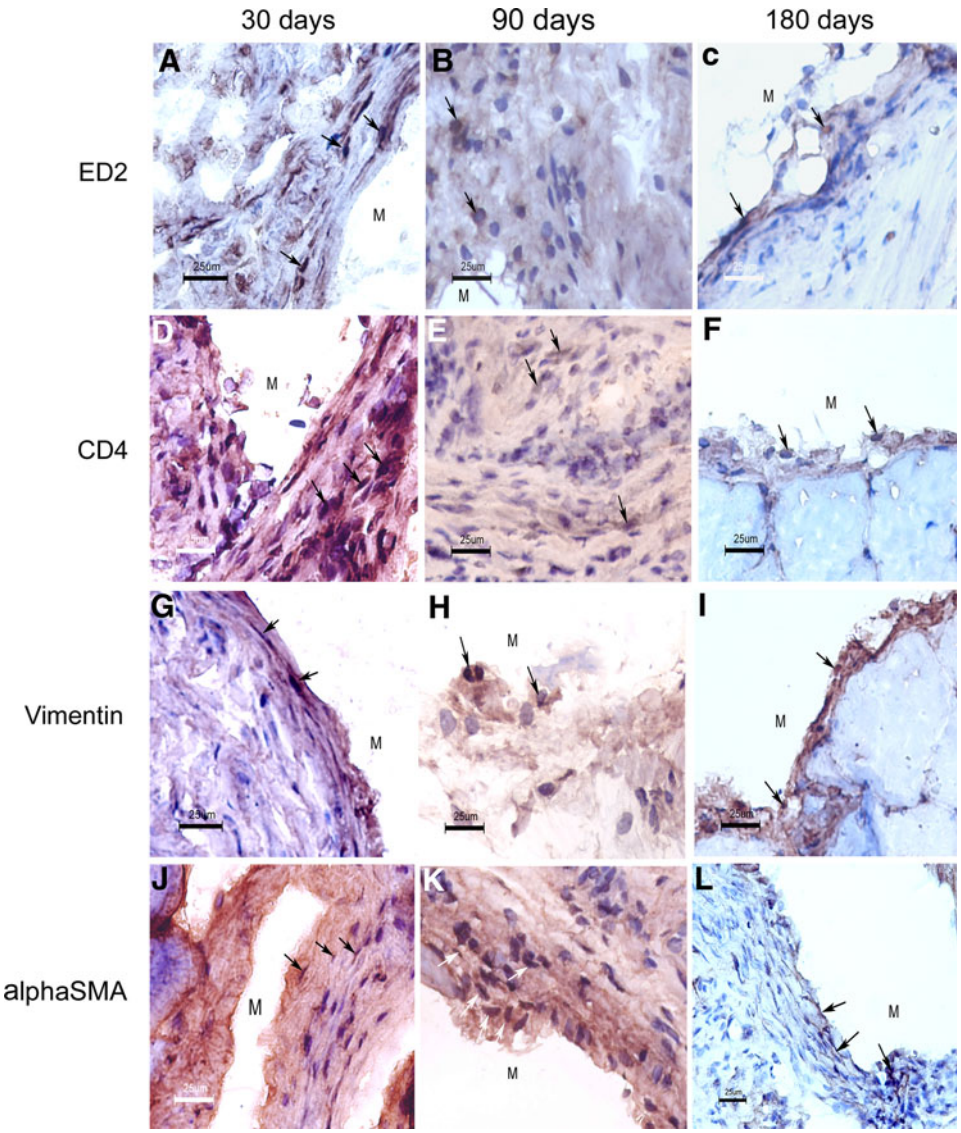

Fig. 3 Light micrographs of immunohistochemical staining for macrophages (ED2) (**a**, **b**, **c**), lymphocytes (CD4) (**d**, **e**, **f**), fibroblasts (Vimentin) (**g**, **h**, **i**) and myofibroblasts (α SMA) (**j**, **k**, **l**) in sections of peri-implant tissue around SE: 30, 90 and 180 days. (M, implant site; ➤, immuno positive cells)

(Fig. 2). In an earlier study, we observed that there was statistically significant difference in the fibrous capsule thickness between both the implants (20 μm around SE and 9 μm around UHMWPE) [12].

Immune cells and cytokines are the master regulators involved in inflammation and repair. In our study we could observe persistence of lymphocytes and macrophages at 180 days around SE, but absent around UHMWPE (Table 2). Numerous studies have related the inflammation and activation of macrophages with release of cytokines, to individual chemical components of the silicone shell [13]. In this study, subpopulations of macrophages were phenotyped using antibodies to ED2. CD 163 or ED2 is a cell surface glycoprotein of 175KD. It is expressed by approximately 50% of peritoneal macrophages, a subset of splenic macrophages and by resident mature macrophages in most other tissues and not expressed by monocytes. Our results are in corroboration with findings of Mancino et al. that macrophage plays an important role in the wound healing around silicone implants and they do persist even at long term [14].

T lymphocytes were identified by using specific antibody that recognizes CD4 cell surface glycoprotein of helper T cells. It was reported that Strong T cell immune response was noted in immunohistochemical studies of retrieved capsule tissue from patients [15]. T cell receptors were also identified in capsular tissue [16]. Flow cytometric studies of patient samples revealed that 89% of implant associated lymphocytes were T cells [17]. In this study, the presence of Th cells around silicone at 180 days in comparison to their absence around UHMWPE, suggests a probable immune response to the silicone bulk material or to released particles from the implant. Dolores et al. have suggested that silicone implant either directly or

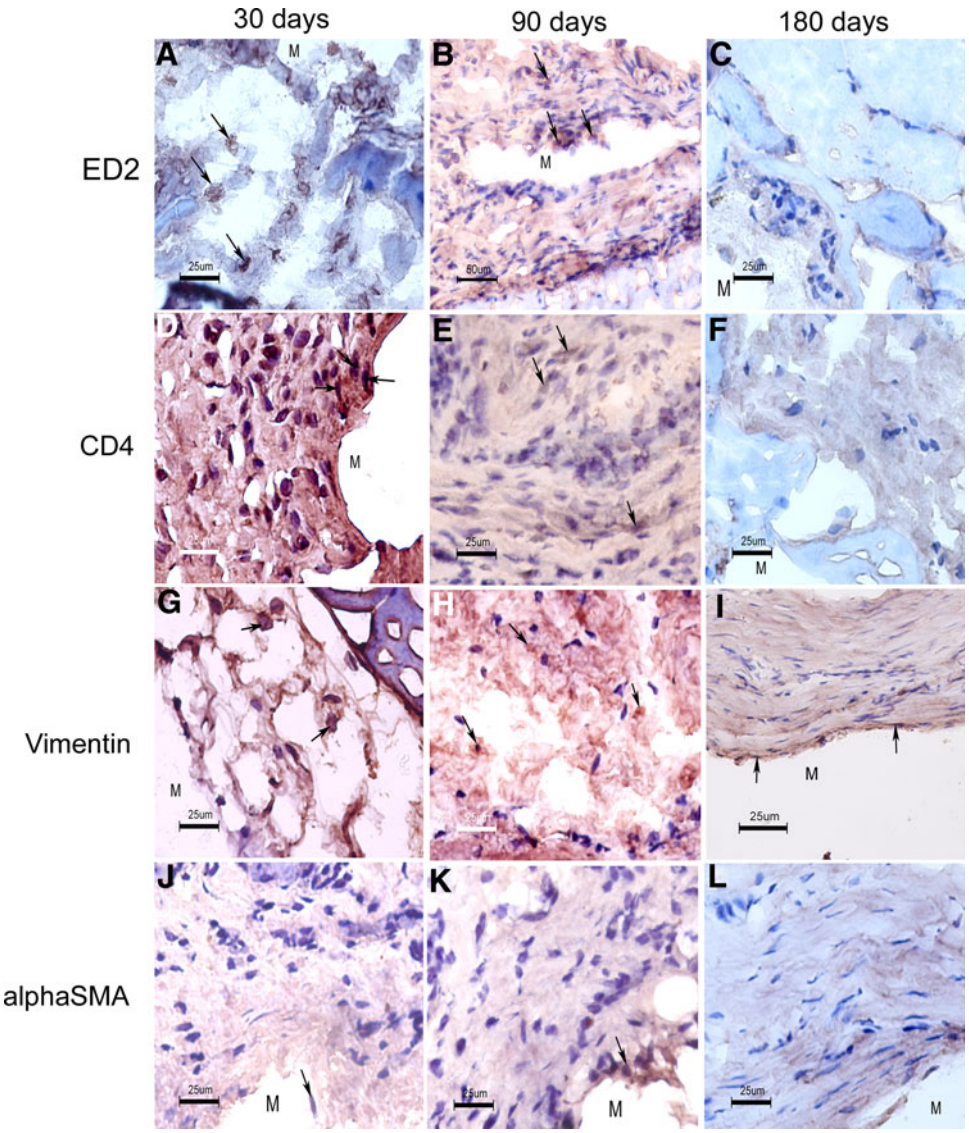

Fig. 4 Light micrographs of immunohistochemical staining for macrophages (ED2) (**a**, **b**, **c**), lymphocytes (CD4) (**d**, **e**, **f**), fibroblasts (Vimentin) (**g**, **h**, **i**) and myofibroblasts (αSMA) (**j**, **k**, **l**) in sections of peri-implant tissue around UHMWPE: 30, 90 and 180 days. (M, implant site; ◥, immuno positive cells)

Table 2 Semi-quantitative evaluation of cells in the peri-implant tissue

Cells	SE			UHMWPE		
	30 days	90 days	180 days	30 days	90 days	180 days
ED2	++++	+++	+	++++	++	Nil
CD4	++++	+++	+++	++++	Nil	Nil
Vimentin	+	++++	++	+	+++	++
αSMA	++	++++	++++	+	++	Nil

indirectly induces a specific T-cell dependant immune response and these lymphocytes may be activated by their interaction with cells of innate immune system [15]. Whereas Jones et al. [18] reported that biomaterials non-specifically stimulated T cell proliferation.

Fibrosis is the response of fibroblasts to stimulation by cytokines; typically produced in chronic inflammation. The main cell types in fibrotic tissue are fibroblasts and myofibroblasts which in chronic inflammation contribute to walling off of the infected area or foreign body [19]. Vimentin positive fibroblasts in the capsular tissue were observed to decrease over time. This may be indicative of cells undergoing apoptosis as in normal wound healing, or to transformation of fibroblasts to phenotypically different cells, myofibroblasts.

Myofibroblasts in wound tissue have been assumed to originate from local recruitment of fibroblasts in the surrounding dermis and subcutaneous tissue [20]. Another possible source being pericytes or vascular smooth muscle cells around vessels in the granulation tissue. Circulating precursor cells, called fibrocytes, migrate to the wound site and contribute to the formation of the myofibroblastic population of granulation tissue [21]. The most prominent marker of fibroblast to myofibroblast transition is αSMA [22]. It has been shown that αSMA expression in stress

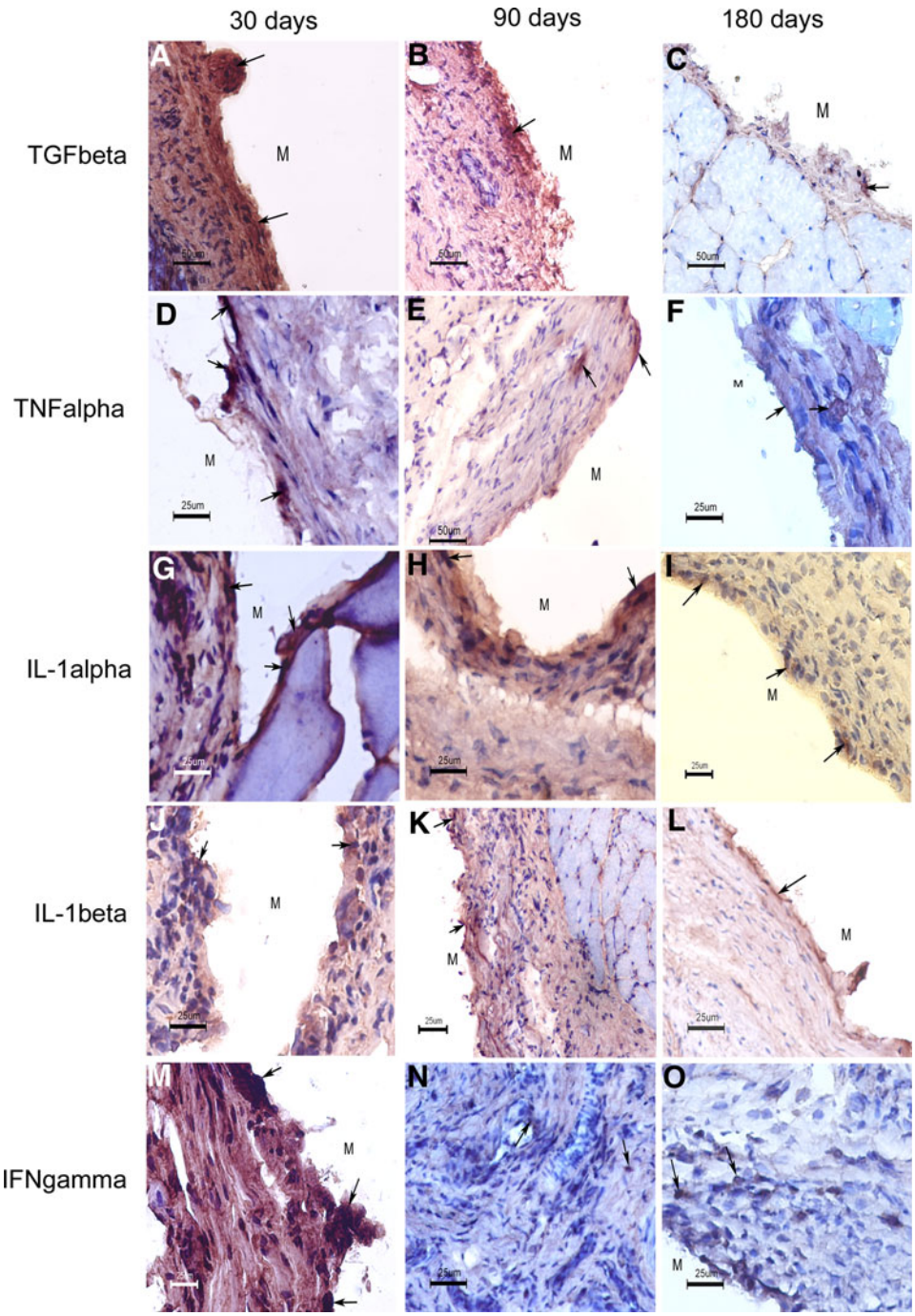

Fig. 5 Immunohistochemical identification of cytokines in peri-implant tissue around SE: 30, 90 and 180 days. (M, implant site; ➶, positive staining)

fibers is essential for the acquisition of a high contractile activity by myofibroblasts, in the absence of any other Smooth muscle specific contractile protein [23]. It was observed that the expression of αSMA positive myofibroblasts increased around SE over time, in comparison to that around UHMWPE as shown in Table 2. In 1978, Rudolph et al. reported contractile fibroblast in fibrous tissue capsules around silicone breast implants [24]. Normally in wound healing, myofibroblasts disappear by apoptosis in later stages [25]. Inappropriate delay of apoptosis, and thus increased survival of activated myofibroblasts during the healing process, may be a factor which leads to excessive scarring in fibrosis [26]. Laitung et al. have found that the large number of myofibroblasts in tissue around tissue expanding devices to be contradictory to the lower capsular contracture usually found with these devices and suggested a long-term animal study to elucidate the early and late phases of scar formation [6].The present investigative

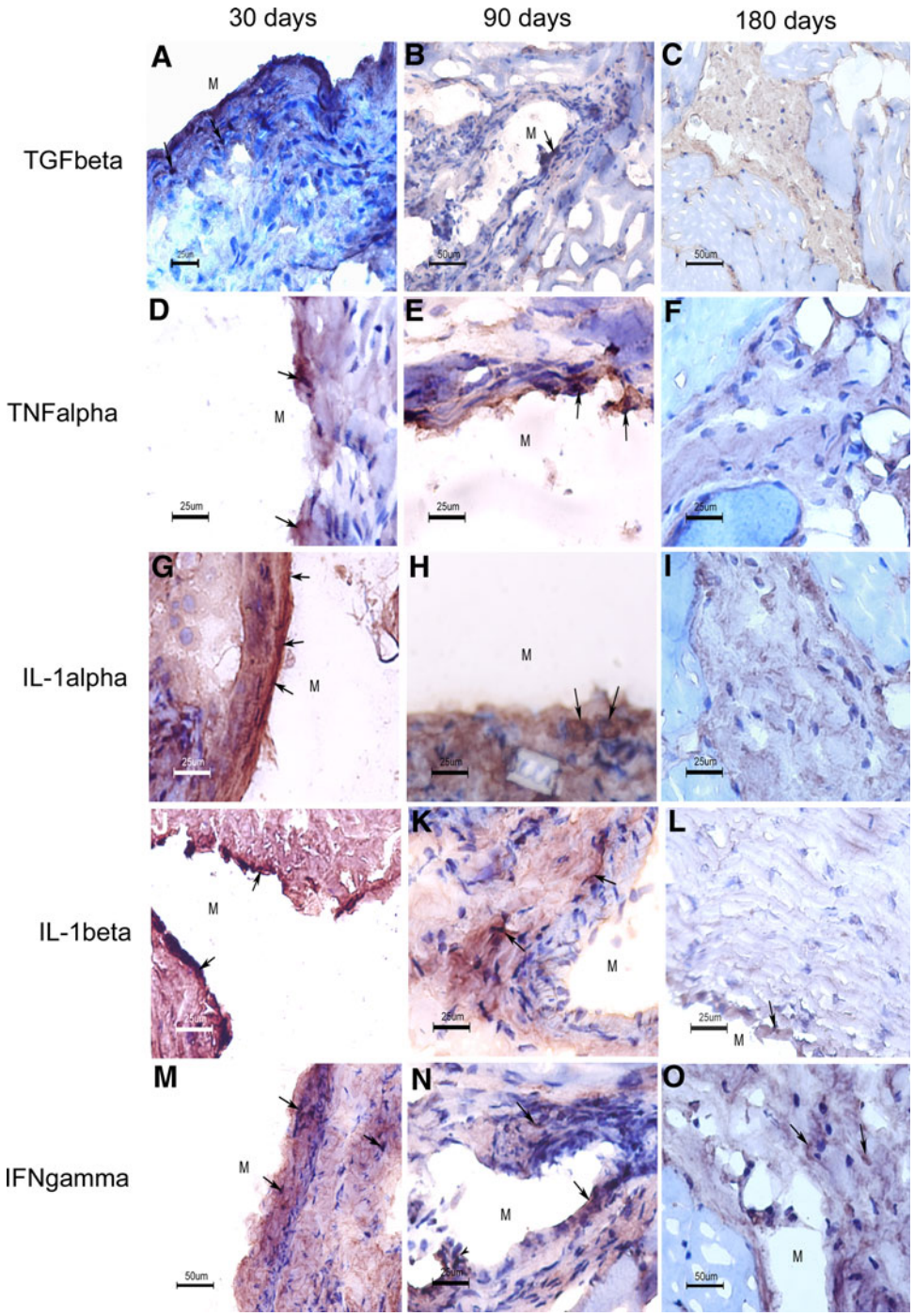

Fig. 6 Immunohistochemical identification of cytokines in peri-implant tissue around UHMWPE: 30, 90 and 180 days. (M, implant site; ➶, positive staining)

study over a span of 180 days in the rat model does establish the persistence of myofibroblasts.

Cytokines released by inflammatory cells are involved through their effect on fibroblasts, in modulation of collagen deposition in the repair phase. In vitro experiments with silicone particles by Granchi et al. have led to a hypothesis that the macrophage ingestion of these particles induces a state of activation leading to release of cytokines with high fibrogenic activity [27]. In this study, TGFβ which is profibrotic, was observed to be initially high with gradual reduction to mild levels at 180 days. This appears contradictory, but is concomitant with the amount of collagen deposited. A similar trend was observed around UHMWPE where the expression was lesser at 90 days and absent at 180 days. The TGFβ family exerts its effects mainly through Smad signal transduction pathways. It was

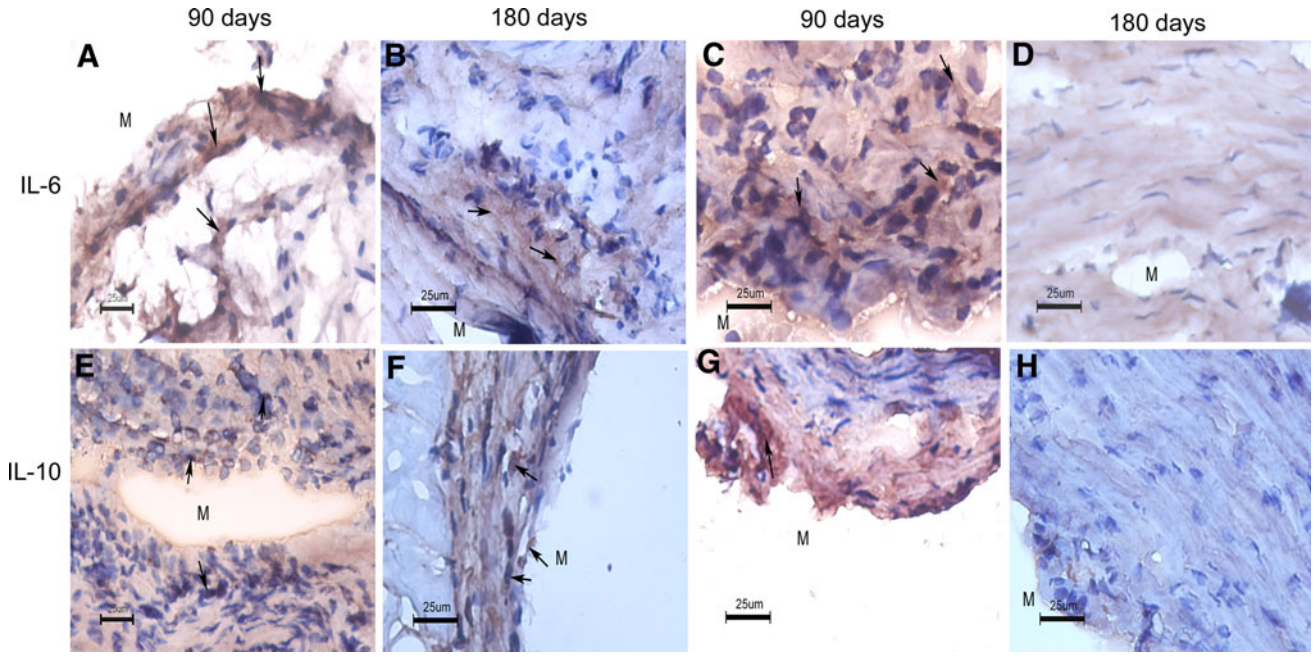

Fig. 7 Immunohistochemical identification of cytokines in peri-implant tissue around SE and UHMWPE: 30, 90 and 180 days. (M, implant site; ↙, positive staining)

Table 3 Semi-quantitative evaluation of cytokines in peri-implant tissue

Cytokines	SE			UHMWPE		
	30 days	90 days	180 days	30 days	90 days	180 days
TGFβ	+++	+++	+	++	+	Nil
TNFα	+++	++	+	++	+	Nil
IL-1α	+++	+++	++	++	++	Nil
IL-1β	++	++	+	+++	+++	++
IFNγ	+++	+	++	++	++	+
IL-6		++	+		+++	Nil
IL-10		+++	+++		+	Nil

reported that TGFβ inhibitor application onto silicone implants prior to implantation have resulted in inhibition of capsular thickness [28].

This is also corroborated with the scant presence of TNFα which is anti-fibrotic. The scant TNFα and negligible TGFβ are both responsible for modulating the collagen thickness. In UHMWPE, both were absent and collagen was thin.

TGFβ and IFNγ exert opposite effects on collagen synthesis [29]. IFNγ which could decrease collagen content in wounds and reduce myofibroblasts also appears to be concomitant with the collagen and myofibroblasts around silicone. Its negligible presence at 90 days and mild at 180 days is concomitant with increase in collagen at 90 days and reduction at 180 days. Around UHMWPE a similar trend could be observed. The increase of IFNγ at 90 days and decrease at 180 days is concomitant with decrease of collagen at 90 days and steady at 180 days. $CD4^+$ T cells which are present at 90 and 180 days have been established as the primary source of IFNγ in healing [30].

Thus it is evident that the fibrogenic cytokines released by macrophages play a significant role in survival of the myofibroblasts in the peri-implant tissue. It was reported that fibroblasts can transform into myofibroblasts through a process regulated by cytokines such as TGFβ, extra cellular matrix molecules such as cellular fibronectin splice variant extra type III domain-A(ED-A) and a mechanically stressed environment [31].

Ultra structural features of these cells are also important in understanding their physiology and function. The data obtained from TEM, substantiated the results from Immunohistochemistry. The excessive fibrosis observed via light microscopy at longer time periods of 90 and 180 days, was confirmed via TEM. Numerous myofibroblasts with ultra structural features of abundant and prominent rough endoplasmic reticulum, modestly developed myofilaments with focal densities (stress fibres) and fibronexus junctions [32] were observed around SE.

Fibrous capsule formation, presence or absence of fibroblasts and myofibroblasts with relation to the levels of inflammatory cytokines at the silicone–tissue interface have not been studied so far in long-term in vitro studies. This study reveals the definite presence of myofibroblasts around silicone implants at 180 days as compared to an

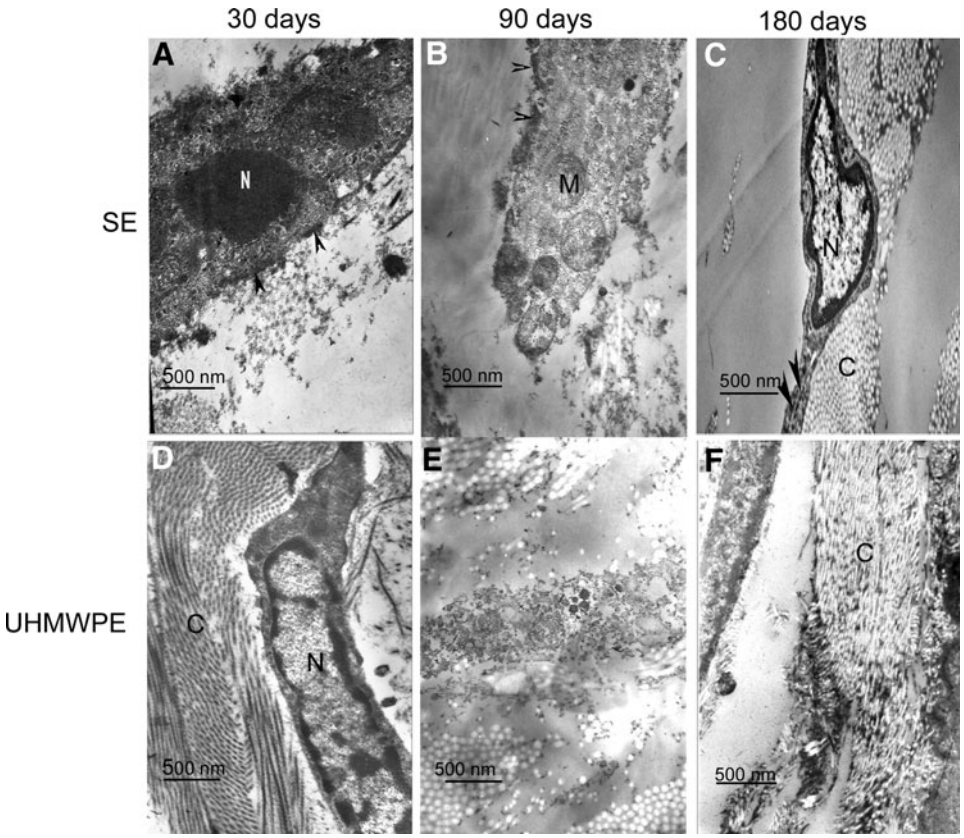

Fig. 8 Transmission electron micrographs of ultra thin sections of peri-implant tissue around SE: 30 days (**a**), 90 days (**b**), 180 days (**c**) and UHMWPE: 30 days (**d**), 90 days (**e**), 180 days (**f**). (C, Collagen; N, nucleus; M, mitochondria; ➤, Fibronexuses and intra cytoplasmic bundles)

absence around UHMWPE. The termination of the acute events with the formation of a fibrous capsule is the result of the delicate balance between pro- and anti-fibrotic proteins. These include pro-fibrotic cytokines TGFβ, CTGF and anti-fibrotic ones like TNFα and IFNγ. Fibroblast to myofibroblast transition begins with the appearance of the protomyofibroblast [33]. Furthermore, myofibroblasts show increased production of collagens I, III, V, fibronectin and proteoglycan proteins [34] thus providing a feedback mechanism in further transformation of fibroblasts to myofibroblasts. The results of this study do emphasize the need for further investigation into the molecular mechanisms of protomyofibroblast and myofibroblast formation around silicone elastomer implants, which would provide information on these target cells for inhibitory therapy in the clinical situation.

5 Conclusions

This study demonstrates a persistence of macrophages, lymphocytes and myofibroblasts around SE when compared to UHMWPE. This difference suggests the role of various immune cells, through their cytokines in regulation of the fibrous capsule formation around silicone implants. Results of this study indicate the need to elucidate the intracellular signaling molecules in the transition of fibroblast to myofibroblasts around silicone elastomer implants.

Acknowledgements The first author thank Council of Scientific and Industrial Research, Govt. of India for the award of research fellowship to carry out this work. The authors acknowledge Director, Sree Chitra Tirunal Institute for Medical Sciences and Technology and Head, Bio Medical Technology Wing for the facilities provided for this study. The authors thank Dr. Annie John for Transmission electron microscopy studies.

References

1. Silverman BG, Brown SL, Bright RA, Kaczmarek RG, Arrowsmith-Lowe JB, Kessler DA. Reported complications of silicone gel breast implants: an epidemiologic review. Ann Intern Med. 1996;124(8):744–6.
2. Kolb SE. The silicone breast implant controversy. Plastikos Plast Reconstr Surg. 2007;1–3.
3. Kamel M, Protzner K, Fornasier V, Peters W, Smith D, Ibanez D. The peri-implant breast capsule: an immunophenotypic study of capsules taken at explantation surgery. J Biomed Mater Res (B). 2000;58(1):88–96.
4. Anderson JM. Biological response to materials. Annu Rev Mater Sci. 2001;31:81–110.
5. Wolfram D, Oberreiter B, Mayer C, Soelder E, Ulmer H, Piza-Katzer H, et al. Altered systemic serologic parameters in patients with silicone mammary implants. Immunol Lett. 2008; 118(1):96–100.

6. Laitung JK, McClure J, Shuttleworth CA. The fibrous capsules around static and dynamic implants: their biochemical, histological and ultrastructural characteristics. Ann Plast Surg. 1987; 19:208–16.
7. James SJ, Pogribna M, Miller BJ, et al. Characterization of cellular response to silicone implants in rats: implications for foreign-body carcinogenesis. Biomat. 1997;18:667–75.
8. ISO 10993:12. Biological evaluation of medical devices-Part 12: sample preparation and reference materials. 2nd ed., 2002-12-15.
9. ISO 10993:6. Biological evaluation of medical devices-part 6: tests for local effects after implantation. 2nd ed., 2007-04-15.
10. Desmouliere A, Guyot C, Gabbiani G. The stroma reaction myofibroblast: a key player in the control of tumor cell behaviour. Int J Dev Biol. 2004;48:509–17.
11. Picha GJ, Goldstein JA. Analysis of the soft-tissue response to components used in the of breast implants: a rat animal model. Plast Reconstr Surg. 1990;87:490–500.
12. Joseph J, Mira M, Mohanan PV. Investigative study of myofibroblasts and cytokines in peri-implant tissue of silicone breast expander by RT-PCR in a rat model. J Biomater Sci Polymer Ed. 2009;0:1–14 (in press).
13. Tavazzani F, Xing S, Waddell JE, et al. In vitro interaction between silicone gel and human monocyte-macrophages. J Biomed Mater Res (A). 2005;72:161–7.
14. Mancino D, Vuotto ML, Minucci M. Effects of a crystalline silica on antibody production to T-dependant and T-independent antigens in Balb/c mice. Int Arch Allergy Appl Immunol. 1984;73: 10–3.
15. Dolores W, Christian R, Harald N, Hildegunde P, Georg W. Cellular and molecular composition of fibrous capsules formed around silicone breast implants with special focus on local immune reactions. J Autoimmun. 2004;23:81–91.
16. TPO' Hanlon, Okada S, Love LA, Dick G, Young VL, Miller FW. Immunohistopathology and T cell receptor gene expression in capsules surrounding silicone breast implants. Curr Top Microbiol Immunol. 1996;210:237–42.
17. Katzin WE, Feng L, Abbuhi M, Klein MA. Phenotype of lymphocytes associated with the inflammatory reaction to silicone gel breast implants. Clin Diagn Lab Immunol. 1996;3(2):156–61.
18. Jones KS. Effects of biomaterial induced inflammation on fibrosis and rejection. Semin Immunol. 2008;20:130–6.
19. Majno G, Joris I. Chronic inflammation. In Cells, tissues and diseases: principles of general pathology. Blackwell Sciences, 1996: 442–70.
20. Ross R, Everett NB, Tyler R. Wound healing and collagen formation. The origin of the wound fibroblast studied in parabiosis. J Cell Biol. 1970;271:4916–22.
21. Gabbiani G. Evolution and clinical implications of the myofibroblast concept. Cardiovasc Res. 1998;38:545–8.
22. Desmouliere A, Rubbia-Brandt L, Abdin A, Walz J, Maciera-Coelho A, Gabbiani G. Alpha smooth muscle actin is expressed in a subpopulation of cultured and cloned fibroblasts and is modulated by gamma-interferon. Exp Cell Res. 1992;201:64–73.
23. Hinz B, Gabbiani G. Cell-matrix and cell-cell contacts of myofibroblasts: role in connective tissue remodeling. Thromb Haemost. 2003;90:993–1002.
24. Rudolph R, Abraham J, Vecchione T, et al. Myofibroblasts and free silicon around breast implants. Plast Reconstr Surg. 1978;62:185–96.
25. Darby I, Skalli O, Gabbiani G. Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Invest. 1990;63:21–9.
26. Desmouliere A, Redrad M, Darby I, Gabbiani G. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. Am J Pathol. 1995;146:56–66.
27. Granchi D, Cavedagna D, Ciapetti G, et al. Silicone breast implants: the role of immune system on capsular contracture formation. J Biomed Mater Res. 1995;29:197–202.
28. Ruiz-de-Erenchun RMD, Herrerias JD, Hontanlla BMD. Use of transforming Growth factor-(beta)1 inhibitor peptide in periprosthetic capsular fibrosis: Experimental model with tetraglycerol Dipalmitate. Plast Reconstr Surg. 2005;116:1370–8.
29. Weng H, Mertens PR, Gresner AM, Dooley S. IFN-γ abrogates profibrogenic TGF-β signaling in liver by targeting expression of inhibitory and receptor Smads. J Hepatol. 2007;46:295–303.
30. Ghosh AK, Yuan W, Mori Y, Chen S, Varga J. Antagonistic regulation of type I collagen gene expression by interferon-gamma and transforming growth factor-beta. Integration at the level of p300/CBP transcriptional coactivators. J Biol Chem. 2001;276:11041–8.
31. Leask A, Abraham DJ. TGF beta signaling and the fibrotic response. FASEB. 2004;18:816–27.
32. Eyden B. Electron microscopy in the study of myofibroblastic lesions. Semin Diagn Pathol. 2003;20(1):13–24.
33. Desmouliere A, Chaponnier C, Gabbiani G. Tissue repair, contraction and the myofibroblasts. Wound Repair Regen. 2005;13: 7–12.
34. Serrini G, Gabbiani O. Mechanisms of myofibroblast activity and phenotypic modulation. Exp Cell Res. 1999;250:273–83.