Titre: Title:	Macrophage response to polyurethane : study of the cytotoxic effects of sterilization cycles and debris
Auteur: Author:	Nan Ma
Date:	2001
Type:	Mémoire ou thèse / Dissertation or Thesis
Référence: Citation:	Ma, N. (2001). Macrophage response to polyurethane : study of the cytotoxic effects of sterilization cycles and debris [Mémoire de maîtrise, École Polytechnique de Montréal]. PolyPublie. https://publications.polymtl.ca/27235/

UNIVERSITÉ DE MONTRÉAL

MACROPHAGE RESPONSE TO POLYURETHANE: STUDY OF THE CYTOTOXIC EFFECTS OF STERILIZATION CYCLES AND DEBRIS

NAN MA

INSTITUT DE GÉNIE BIOMÉDICAL

ÉCOLE POLYTÉCHNIQUE DE MONTRÉAL

MÉMOIRE PRÉSENTÉ EN VUE DE L'OBTENTION

DU DIPLÔME DE MAÎTRISE ÈS SCIENCES APPLIQUÉES (M.Sc.A)

(GÉNIE BIOMÉDICAL)

OCTOBRE 2001

UNIVERSITÉ DE MONTRÉAL

ÉCOLE POLYTECHNIQUE DE MONTRÉAL

Ce mémoire intitulé:

MACROPHAGE RESPONSE TO POLYURETHANE: STUDY OF THE CYTOTOXIC EFFECTS OF STERILIZATION CYCLES AND DEBRIS

présenté par: MA Nan

en vue de l'obtention du diplôme de: Maîtrise ès sciences appliquées

a été dûment accepté par le jury d'examen constitué de:

M. SAVARD Pierre, Ph.D., président

M. YAHIA L'Hocine, Ph.D., membre et directeur de recherche

Ms. TABRIZIAN Maryam, Ph.D., membre et codirectrice de recherche

Ms. HUK Olga, MD, M. Sc.A., membre et codirectrice de recherche

M. MERHI Yahye, Ph.D., membre

Il n'y a qu'une frontière,

c'est celle de nos connaisances,

et elle est mobile!

-J. M. STERKERS

À mon mari et ma fille

Remerciements

Je voudrais remercier mon directeur de recherche, le professeur L'Hocine Yahia, pour m'avoir acceptée au sein du Groupe de Recherche en Biomécanique et Biomatériaux, ce qui m'a permis de réaliser ma maîtrise dans le domaine fascinant des biomatériaux.

J'aimerais exprimer ma gratitude à ma codirectrice de recherche, le Docteur Maryam Tabrizian. J'apprécie la compréhension, les conseils et la patience dont elle a fait preuve envers moi pendant les deux longues années qu'a duré cette maîtrise. Ses connaissances abondantes dans le domaine de la stérilisation et de la chimie m'ont guidée à travers ce travail intéressant mais toutefois difficile.

Je tiens également à remercier vivement mon autre codirectrice de recherche, le Docteur Olga Huk qui m'a permis de m'intégrer au sein de ses laboratoires respectifs au Lady Davis Institute et à l'Hôpital général juif.

Je voudrais exprimer ma gratitude et ma reconnaissance envers le Docteur Alain Petit, qui m'a toujours offert son aide avec zèle et sans hésitation quand j'en avais besoin durant ces deux années.

Je tiens à remercier le professeur Pierre Savard, qui m'a introduit au domaine des biomatériaux. Il m'a constamment offert son aide, ses conseils et son encouragement et a aussi accepté d'évaluer mon travail en faisant partie de mon jury.

Je voudrais exprimer ma gratitude au Dr Yahye Merhi qui a gracieusement accepté d'évaluer mon travail en faisant partie du jury.

Je tiens à remercier Caroline Demers pour sa présence et son aide qui m'ont fourni du courage et des idées originales et pratiques. J'apprécie surtout ses talents de linguiste accomplie qui ont sauvé mon mémoire de fautes grammaticales et orthographiques.

Je tiens également à remercier toutes les personnes qui m'ont apporté une aide scientifique ou technique lors de ce travail, notamment:

Madame Stefania Polizu pour son aide dans les analyses de microscopie électronique balayage et dans l'assemblage de mon mémoire.

Benjamin Thierry pour l'analyse de la spectroscopie infrarouge.

Sophie Lerouge pour m'avoir offert les cathéters d'électrophysiologie.

Louise Clément et Diane Giroux pour leur sympathie, leur aide indispensable.

Tout le personnel et les étudiants du GRBB.

Enfin, je désire remercier particulièrement mon mari et ma fille pour leur compréhension, leur encouragement, et leur soutien inconditionnel au long de chaque étape de ma maîtrise.

Résumé

Aujourd'hui les polyuréthanes (PUs) constituent un des meilleurs choix de biomatériaux pour la fabrication d'appareils médicaux dont les applications exigent la conformité aux tissus souples ou cardiovasculaires. Pourtant, l'interaction entre les polyuréthanes et l'environnement biologique dans lequel ils sont implantés n'est pas encore entièrement comprise. Donc, les applications biomédicales du polyuréthanes sont largement limitées par notre manque de connaissance à ce sujet. Même si les polyuréthanes sont normalement considérés comme non toxiques, plusieurs études ont montré qu'ils sont instables et émettent des substances toxiques lors de leur contact avec l'environnement biologique. Il est bien connu que la biocompatibilité est l'une des plus importantes propriétés biomédicales requises dans les applications médicales. Ainsi, la toxicité des polyuréthanes est devenue un problème extrêmement important dans le développement et les applications de ces derniers. Ce mémoire se concentre sur la réponse cellulaire aux matériaux à base de polyuréthane commercialement disponibles. Puisqu'il a déjà été démontré que les macrophages jouent un rôle crucial dans la biodégradation et la biostabilité des polyuréthanes, nous avons étudié l'effet des polyuréthanes sur la viabilité et l'activité des macrophages. Par suite, nous avons formulé l'hypothèse que les débris et/ou les produits relargés par les matériaux et appareils à base de polyuréthanes peuvent attirer et activer les macrophages au site de l'implantation, résultant ainsi dans le développement d'une inflammation. Par conséquent, ces produits relargés peuvent aussi

changer l'interaction du tissu et de l'implant à base de polyuréthanes. Cela pourrait ensuite causer la dégradation de l'implant et même menacer la santé des patients. Le but général de ce travail de maîtrise est d'analyser la biocompatibilité des appareils à base de polyuréthanes de degré médical selon leur cytotoxicité envers les macrophages ainsi que l'action des macrophages.

Les études ont été réalisées *in vitro*. La ligne cellulaire de macrophage J774 fut utilisée pour évaluer les réactions inflammatoires. La viabilité des cellules fut mesurexaminée par l'exclusion du Trypan bleu. La présence du médiateur inflammatoire TNF-α fut analysée par le test ELISA.

La première partie de notre étude se présente sous la forme d'une étude sur des effets cytotoxiques potentiels des particules de polyuréthanes dans le but d'identifier les facteurs qui influencent la stabilité des polyuréthanes. Nous avons testé des polyuréthanes commercialement disponibles, possédant les mêmes composants mais de structures différentes. Les caractéristiques des particules furent analysées par microscopie électronique à balayage (MEB), la spectroscopie infrarouge à réflexion (ATR-FTIR), la résonance magnétique nucléaire (RMN) et la chromatographie à gaz combinée à la spectroscopie de masse (CG-SM). Notre étude démontre que la morphologie de nos particules de polyuréthanes est semblable à celle observée *in vivo*. Les trois particules que nous avons utilisées possèdent la même composition mais avec des proportions différentes de segments souples et durs. L'interaction de ces particules avec les cellules

indique que la concentration et le type de particules sont deux paramètres importants qui influencent la réponse des macrophages. Les résultats ont révélé que l'induction de la cytotoxicité et l'activation des macrophages dépendent de la dose de particules, avec des effets significatifs à des concentrations supérieures à 50 μg/ml. La cytotoxicité est aussi reliés aux proportions de segments souples et durs de polyuréthanes Plus la structure chimique du polyurethanes est rigide, plus la cytotoxicité est élevée.

Dans la deuxième partie de l'étude, nous avons examiné les effets toxiques potentiels des cathéters électrophysiologies re-stérilisés à base de polyuréthanes sur la mortalité et l'activité cellulaire. Nous avons analysé les effets de différentes techniques de stérilisation de même que le nombre de cycles de stérilisations, sur les macrophages J774. L'analyse de cytotoxicité dévoile que les méthodes de stérilisation présentement utilisées n'ont aucun effet toxique sur les cathéters électrophysiologies après plusieurs traitements. Nos résultats suggèrent aussi que parmi toutes les méthodes utilisées, l'oxyde d'éthylène et le Sterrad 100S® semblent être les meilleurs choix pour la stérilisation des cathéters à base de polyuréthanes

En conclusion, notre étude indique que la réponse des macrophages aux débris de polyuréthanes dépend de la concentration des particules de polyuréthanes De plus, la proportion des segments souples et durs est un paramètre important affectant la réponse des macrophages aux polyuréthanes. Cette étude suggère que la réduction des débris et le choix approprié de la longueur du segment dur devraient être considérés pour améliorer

les appareils à base de polyurethanes présentement disponibles. La restérilisation des cathéters à base de polyuréthanes dans des conditions hospitalières n'a aucun effet cytotoxique significatif à court terme·sur les macrophages J774.

Abstract

Polyurethanes have been increasingly accepted as one of the best choices of biomaterials for the fabrication of medical devices in applications requiring compliance with soft or cardiovascular tissues. However, the interaction between polyurethanes and the biological environment in which they are implanted is not yet fully understood. Therefore, polyurethane's biomedical applications are limited by our lack of knowledge in this field. Although polyurethanes have been considered as non-toxic, several studies have shown that they are unstable and release toxic substances when they come in contact with the biological environment. It is well known that biocompatibility is one of the most important biomaterial properties required in medical applications. Therefore, the toxicity of polyurethanes becomes an extremely important issue in their development and their applications. The study of this thesis was focused on the cellular response to commercially available polyurethane materials.

Since it has been demonstrated that macrophages play a crucial role in the biodegradation and biostability of polyurethanes in several devices, we hypothesized that debris and/or leachables that are released from polyurethane materials and devices may attract and activate macrophages at the site of implantation, resulting in inflammation. This is also provoke changes in the subsequent interaction of the tissue and the polyurethane-based implant, causing degradation and even failure of the devices, which may threaten the safety of patients. The general goal of the present work was to measure the macrophage

response to medical degree polyurethanes in terms of macrophage cytotoxicity and activation.

The studies were performed in an *in vitro* model. The J774 macrophage cell line was used to determine the inflammatory reactions. The cell viability was measured by Trypan Blue exclusion. Inflammatory mediator TNF-α was measured by ELISA test.

In the first part of this study, we investigated the potential cytotoxic effects of polyurethane particles for the purpose of identifying factors that influence polyurethane material stability. We tested different commercially available polyurethanes that possess the same moiety but different structures. The characteristics of the particles were analyzed by Scanning Electron Microscopy (SEM), Fourier Transfom Infrared-Attenuated Total Reflectance Spectroscopy (FTIR-ATR), Nuclear Magnetic Resonance (NMR) and Gas Chromatography-Mass Spectroscopy (GC-MS). Our study showed that the morphology of our polyurethane particles is similar to those observed *in vivo*. The three particles used possessed the same composition but different hard/soft segment ratios. The interaction of these particles with cells showed that particle concentration and type are two important parameters influencing macrophage response. Results demonstrated that the induction of macrophage cytotoxicity and activation was dose-dependent with significant effects at concentrations higher than 50 μg/ml particles. Cytotoxicity was also related to the ratio of hard/soft segments of polyurethane: the harder the chemical structure of the polyurethane, the higher the cytotoxicity.

The second part of our study measured the potential effects of resterilized polyurethane-based electrophysiology catheters on cell mortality and activity. We analyzed the effect of different sterilization techniques, as well as the number of sterilizations, on J774 macrophages. Our results demonstrated that currently used sterilization methods have no significant toxic effects on polyurethane-based electrophysiology catheters even after multiple treatments. Our results also suggest that among these methods, Ethylene oxide (EtO) and Sterrad 100S® seemed the most suitable for the resterilization of polyurethane-based catheters.

In conclusion, our study demonstrated that macrophage response to polyurethane debris depends on the concentration of the polyurethane particles. The ratio of hard/soft segments is also an important parameter affecting macrophage response to polyurethane. This study suggests that the reduction of material debris and the correct choice of the hard segment length should be helpful in improving current polyurethane-based device design. Furthermore, the resterilization of polyurethane-based catheters under hospital condition has no significant cytotoxic effect on J774 macrophages.

Effet des polyuréthanes sur les macrophages

Les polyuréthanes (PUs) furent découverts en 1937 et introduits au sein de la communauté biomédicale en 1967. Après trente ans de développement, le PU est reconnu comme l'un des biomatériaux les plus biocompatibles et, de plus en plus reconnu comme étant l'un des meilleurs choix pour la fabrication des prothèses médicales, particulièrement pour les prothèses cardiovasculaires et les tissus conjonctifs (Lamba *et al.*, 1998; Pinchuk L., 1994; Szycher, 1991; Zdrahala 1996). Cependant, différents problèmes surviennent lors des applications chirurgicales ainsi que dans les expériences *in vivo* et *in vitro* (Parins *et al.*, 1981; Pinchuk, 1994; Szycher, 1985). À cause de la dégradation des matériaux de PU, beaucoup de prothèses fabriquées à base de ceux-ci ont subi des échecs en clinique. Des fissures en surface ont aussi été observées dans des implants de PU. Ces effets indésirables réduisent la fonctionnalité des prothèses et, parfois, peuvent mettre en péril la vie du patient. L'instabilité biologique à long terme limite donc les applications des PUs en clinique (Meijs *et al.*, 1993; Stoke *et al.*, 1995).

Plusieurs études ont démontré qu'une réponse inflammatoire chronique associée à l'activation des macrophages semble être l'un des facteurs primordiaux du déclenchement de la biodégradation des PUs (Anderson *et al.*, 1988; Labow *et al.*, 1999). Ce phénomène a été attribué à des interactions cellulaires à la surface des matériaux implantés (Zhang et al., 1994; 1996; Zhao *et al.*, 1990). Cependant, le mécanisme de

l'activation des macrophages en réponse aux surfaces et aux débris des PUs n'est pas encore clairement compris.

Le but général de ce travail de maîtrise était de comprendre la réponse des macrophages aux différents types de PUs utilisés dans le domaine biomédical. Le travail a été divisé en deux parties. La première partie a consisté à évaluer les effets cytotoxiques des particules de PU sur la lignée cellulaire de macrophages J774. La seconde partie a évalué les effets cytotoxiques potentiels (mortalité et sécrétion de TNF-α) des cathéters électrophysiologiques à base de PU restérilisés, sur ces mêmes macrophages

Cytotoxicité des particules de PU

Comme l'identification des débris de PU dans les tissus des patients est limitée et que leur isolation pourrait causer leur altération, nous avons procédé à la préparation *in vitro* de particules de PU. Trois types de PU commercialement disponibles ont été utilisés: le Pellethane® 2363 80ABA40 (PL), le Técothane® TT2065DB40 (TC65) et le Técothane® TT2085AB40 (TC85). Une version modifiée de la méthode de solvent évaporé à été mise au point afin d'obtenir des particules de taille phagocytable tout en assurant la reproductibilité des échantillons (Sato *et al.*, 1992). Des particules de moins de 5 μm furent donc utilisées comme produits de relâche ou de dégradation des PUs pour l'évaluation de l'effet cytotoxique des PUs sous conditions controlées. La microscopie électronique à balayage (SEM) a démontré que la forme des particules était irrégulière

(ovale, ronde) et que leurs tailles variaient de 0.8 à 5.0 µm. La spectroscopie infrarouge à réflexion atténuée (ATR- FTIR) nous a permis de voir que ces polymères possédaient la même composition générique mais étaient différentes par les proportions de leurs segments souples et durs. Les analyses de Résonnance Magnétique Nucléaire (RMN) ont démontré que les proportions de segments durs aux segments souples étaient de 37%, 39% et 58% pour le PL, le TC85 et le TC65, respectivement.

La cytotoxicité des particules a été mesurée par contact direct. Les macrophages J774 furent ainsi incubés à 37°C pendant 24 ou 48 h avec les différentes particules de PU à des concentrations de 10 à 100 µg/ml. L'exclusion du bleu de trypan fut utilisée pour mesurer la mortalité cellulaire. Finalement, l'activité des macrophages a été quantifiée en mesurant la sécrétion de TNF-α par test ELISA.

Les résultats démontrent que les particules de PU ont affecté la viabilité et l'activité des macrophages J774. En effet, le pourcentage de mortalité a varié de 1 à 15% en fonction du type et de la concentration des particules. Plus spécifiquement, la mortalité a varié de 1 à 4% avec les particules de PL, de 2 à 10% avec les particules de TC85, et de 4 à 15% avec les particules de TC65. Le temps d'incubation (24 ou 48 h) ne semble avoir eu aucun effet sur la viabilité des cellules. Les particules de PU ont aussi influencé la croissance cellulaire. Ainsi, le nombre de cellules a augmenté de 32% et 67% après 24 heures et 48 heures, respectivement, dans les incubations témoins. Nous avons cependant observé une diminution significative du nombre de cellules après 24 h d'incubation avec

100 µg/ml de PL (34%), TC85 (33%) et de TC65 (53%). Cet effet a été moins marqué après 48 h, avec une augmentation du nombre de cellules de 10 et 7% avec les particules de PL et de TC85, respectivement, alors que le nombre de cellules a diminué de 23% avec le TC65.

Les particules de PU ont aussi stimulé la sécrétion de TNF-α par les macrophages. La concentration a augmenté dans toutes les conditions expérimentales et a varié avec la concentration et le type de particules. Après 24 h d'incubation avec 100 µg/ml de particules de PL, TC85 et TC65, la sécrétion de TNF-α était 4, 5, et 7 fois supérieure à celle du témoin. L'augmentation de la sécrétion de TNF-α peut augmenter l'habilité phagocytaire des macrophages. Cela favoriserait donc l'accumulation des macrophages et d'autres cellules au site d'implantation afin d'alimenter davantage la réponse inflammatoire. Ainsi, la réponse inflammatoire chronique observée autour des implants de PU serait due, en partie, à la relâche à long terme des composantes chimiques des PUs *in vivo*.

En résumé, les deux paramètres qui influencent principalement la viabilité des macrophages sont la concentration et le type de particules, la cytotoxicité du Técothane® étant plus élevée que celle du Pellethane®. La concentration et le type de particules ont aussi influencé la croissance des cellules. La seule différence entre les particules réside au niveau des proportions de leurs segments souples et durs. La réponse des macrophages aux particules était TC65 > TC85 > PL, ce qui est le même ordre que la proportion des

segments durs. Ceci suggère fortement une cytotoxicité associée à la présence des segments durs.

Cytotoxicité des cathéters restérilisés

Pour des raisons économiques, la réutilisation des cathéters après leur restérilisation est fortement envisagée de nos jours (Brownw et al., 1997; Conseil d'évaluation des technologies de la santé du Québec, 1994; Lisa, 1995). Cependant, la réutilisation des cathéters peut conduire à des effets indésirables, allant de l'infection à la modification de la surface du cathéter, de la cytotoxicité jusqu'au bris du cathéter. La formation de résidus toxiques sur la surface du cathéter est ainsi un résultat probable du processus de stérilisation (Goldman *et al.*, 1997; Nair, 1995; Shntani *et al.*, 1995). D'autre part, la stérilisation peut changer la surface du cathéter et causer la dégradation de ce dernier (Darby *et al.*, 1978; Jayabalan *et al.*, 1997; Mazzu *et al.*, 1984). Des composantes chimiques des PUs ont été retrouvées à la surface des cathéters après stérilisation. Ces produits potentiellement toxiques peuvent nuire à la santé du patient. Une meilleure connaissance de cette toxicité aiderait à assurer la sécurité des patients. À notre connaissance, aucune étude de l'effet de la stérilisation sur la toxicité cellulaire des cathéters n'a été effectuée. La présente étude visait donc à tester l'effet cytotoxique de la stérilisation répétée des cathéters de PU sur les macrophages.

Nous avons utilisé des cathéters électrophysiologique en PU dit à usage unique, produits par la compagnie *Cordis Corporation* (Floride, É-U). Trois méthodes de stérilisation furent utilisées dans les conditions standard des hôpitaux: Les stérilisations à la vapeur, c'est-à-dire l'autoclave, et à l'oxyde d'éthylène (EtO) furent effectuées à l'Hôpital Général Juif de Montréal, alors que le Sterrad-100S® fut effectué à l'Hôpital Charles Lemoyne de Greenfield Park. Nous avons analysé l'effet des différentes techniques de stérilisation et du nombre de stérilisations (1 ou 10 cycles) sur la cytotoxicité des cathéters en utilisant une méthode d'extraction qui suit les normes du ISO 10993-5:1993. Les cathéters furent incubés dans un milieu RPMI supplé avec 5% de sérum de boeuf fétal à 37°C pendant 72 heures dans une proportion de 1.0 cm de cathéter/ml. Ces solutions d'extraction furent utilisées tout de suite après leur préparation comme milieu d'incubation pour les macrophages J774 (500 000 cellules/ml) pendant 24, 48 ou 72 h. L'exclusion du bleu de trypan a été utilisée pour évaluer la cytotoxicité alors qu'un test ELISA a été utilisé pour mesurer la sécrétion du TNF-α.

Nos résultats ont révélé un changement morphologique important dans le cathéter stérilisé par l'autoclave tandis que les cathéters stérilisés par l'EtO et par le Sterrad 100S® n'ont démontré aucune caractéristique différente de celles des cathéters témoins. Les extraits de ces cathéters stérilisés ont augmenté la mortalité cellulaire de 1 à 10% au-dessus de celle des témoins. Deux paramètres ont influencé cette réponse cellulaire: (1) Le nombre de cycles de stérilisation (plus il y a de cycles, plus la mortalité est élevée.); (2) La méthode de stérilisation. La stérilisation à l'autoclave a causé la plus grande

augmentation de mortalité, possiblement due à une augmentation de la relâche de substances chimiques des PUs à la surface des cathéters lors du processus de stérilisation.

Nous avons aussi observé que le nombre total de cellules était moindre dans les extraits de cathéters que dans les milieux témoins. Cette diminution dans le nombre de macrophages pourrait s'expliquer par l'effet inhibiteur des résidus chimiques produits par la stérilisation ou par des produits de dégradation des PUs. Il n'y avait cependant aucune différence significative entre 1 et 10 cycles de stérilisation et entre les méthodes de stérilisation sur les nombres de cellules. Enfin, la méthode et le nombre de cycles de stérilisation n'ont eu aucun effet sur la sécrétion de TNF-α. Ainsi, nous considérons que l'augmentation de la mortalité cellulaire par rapport à la restérilisation n'est pas associée à une augmentation de l'activité des macrophages. Ces resultats suggèrent que la réponse inflammatoire response est moins forte.

Des effets adverses de l'EtO ont déjà été rapportés par plusieurs études. Donc, le niveau de résidus d'EtO doit être gardé le plus bas possible (Ferrell *et al.*, 1997; National Toxicology Program. Toxicology and carcinogenesis studies of ethylene oxide). Dans notre étude, une période d'aération de 12 h fut allouée entre chaque cycle de stérilisation. Après les dix cycles de stérilisation, une période supplémentaire d'aération de 14 jours fut respectée. Nos résultats ont indiqué qu'après un cycle de stérilisation par l'EtO, il n'y a eu aucun effet cytotoxique significatif sur les macrophages (4% de mortalité). La mortalité a atteint cependant 8% ($p < 0.05$) après dix cycles de stérilisation par l'EtO.

Nous supposons qu'une certaine quantité de résidus chimiques s'est accumulée sur la surface des cathéters suite aux processus de stérilisation par l'EtO.

Les cathéters qui ont été stérilisés par le Sterrad 100S® révèlent un niveau de cytotoxicité comparable à celui des cathéters stérilisés par l'EtO. Dix cycles de stérilisation par la méthode Sterrad 100S® ont produit aussi un niveau de mortalité cellulaire légèrement plus élevé qu'après un cycle de stérilisation (8% *vs* 5%). Cette augmentation, quoique non significative, pourrait être due à une combinaison des effets des résidus chimiques et des produits de dégradation des PUs car le peroxyde d'hydrogène (H_2O_2), un des produits utilisés dans la stérilisation par le Sterrad 100S®, peut oxyder les PUs. La stérilisation par plasma utilisant le H_2O_2 fut développée récemment et peu d'études ont évalué ses effets toxiques potentiels.

En résumé, nos résultats suggèrent que le risque de cytotoxicité associé à la re-stérilisation des cathéters d'électrophysiologie à base de PU demere cependant faible.

Conclusion et Perspectives

Même si les PUs continuent d'être des choix attrayants pour des applications biomédicales, de nouveaux efforts devraient être faits pour mieux comprendre les mécanismes d'échec afin d'améliorer la qualité des implants. La stabilité des biomatériaux implantés ne peut être prédite que si les interactions entre les cellules

inflammatoires et le biomatériau sont bien comprises. La première partie de notre projet a clairement indiqué qu'il existe une relation entre la structure chimique des PUs et leur potentiel cytotoxique. Même si plusieurs études ont déjà démontré la toxicité des PUs, très peu ont regardé les proportions des segments durs et souples sur la réponse cellulaire. Nos résultats suggèrent qu'il serait possible d'abaisser l'activation des macrophages en sélectionnant adéquatement la proportion des segments durs et souples dans les implants de PU.

D'autres travaux seront cependant nécessaires afin de mieux comprendre le processus de phagocytose des particules de PU. Ceci pourrait être réalisé par la cytométrie en flux qui constitue un outil très performant et quantitatif pour ce genre d'études. L'effet des particules de PU sur la prolifération cellulaire devra aussi être analysé par des méthodes plus spécifiques. Il apparait également très important que l'analyse de la relation entre les proportions de segments durs et souples soit poussée plus loin. Finalement, l'analyse de la cytotoxicité de tous les nouveaux PUs devrait être réalisée de façon similaire à la présente étude.

La deuxième partie de la présente étude suggère qu'une stérilisation adéquate des cathéters électrophysiologiques à base de PU ne présente qu'un très faible risque de cytotoxicité et qu'il est très improbable que suffisamment de résidus chimiques soient formés à la surface des cathéters pour nuire à la santé des patients. Ceci pourrait être utile aux agences gouvernementales pour la standardisation de la restérilisation et de la

réutilisation des cathéters cardiaques. Des travaux plus approfondis sont cependant requis pour étudier l'effet toxique produit par le traitement complet des cathéters, incluant donc le nettoyage et la stérilisation.

Table of Contents

List of the Tables

List of the figures

List of abbreviations

AAMI	Association for the Advancement of Medical Instrumentation
BD	1,4-butanedial
cm	Centimeter
DAMCH	Diaminocyclohexane
DHEA	Dehydroepiandrosterone
EDA	Ethylene diamine
ELISA	Enzyme-linked immunosorbent assay
EtO	Ethylene oxide
FDA	Food and Drugs Administration
FTIR-ATR	Infrared-Attenuated Total Reflectance spectroscopy
GC-MS	Gas Chromatography-Mass Spectroscopy
g	Gram
HMDI	Hydrogenated methylene diisocyanate
HMEC	Hezamethylene carbomate
H_2O_2	Hydrogen peroxide
µg	Micro-gram
MDA	4,4' –methylene dianiline
MDI	4,4 -methylene bisphenyl diisocyanate
MEB	Microscopie Électronique à Balayage

ml	Milliliter
NMR	Nuclear Magnetic Resonance
PDMS	Poly(dimethyl siloxane)
PL	Pellethane® 2363A80ABA
PTFE	Polytetrafluoreethylene
PTMO	Polytetramethylene oxide
PUs	Polyurethanes
PVC	Polyvinyl chloride
rpm	Revolution per minute
SEM	Scanning Electron Microscopy
TC65	Tecothane® TT 2065DB40
TC85	Tecothane® TT 2085AB40
TDA	2,4 -toluene diamine
TDI	2,4 -toluene disocyanate
TNF-α	Tumor necrosis factor alpha

CHAPTER 1. INTRODUCTION

Since their introduction in biomedical applications, polyurethanes (PUs) have occupied a considerable and significant place in the making of prostheses and implants because of their excellent mechanical properties and their relative biocompatibility (Lamba *et al.*, 1998; Pinchuk L., 1994). PU-based prostheses and devices range from catheters to artificial organs, including vascular grafts. Their contribution to the improvement of the quality of many human lives has been significant (Zdrahala 1996; 1999).

Three to four million Americans suffer from heart failures each year. Of these, approximately 70,000 need a heart transplant. However, only 4,000 donor organs are available. Meanwhile, the patients who do not get a transplant wait for it and many die while waiting. Moreover, there is a great demand for synthetic materials to repair and replace damaged tissues and organs. The materials used for these applications are limited because they must imitate the properties of such replaced tissue or organ as closely as possible: elasticity, flexibility, high tensile strength and good abrasion resistance. They must be free of any toxicity and be biocompatible. Medical grade PUs are the most promising biomaterials to meet these requirements.

Despite their numerous qualities, PUs have not yet reached the status of natural tissue replacements. Indeed, the best PU-based implants have been limited to uncertain long-

term biostability when exposed to the harsh environment of the human body. The major cause of PU-based implant failure is biodegradation. Biodegradation causes surface cracking of prosthesis surface, releasing degradation products. The surface fissuring followed by the deep cracking can result in loss of implant function, which may even threaten the patient's life (Carson *et al.*, 1996). The mechanisms of PU device failure will be discussed in Chapter 2.

Although extensive studies focused on the mechanism of failure of PU-based devices, it is still uncertain what molecular and cellular responses are critical in this process. Nevertheless, the role of biomaterials releasing substances that activate inflammatory cells appears to be a primary factor (Hinrichs *et al.*, 1992; Xi *et al.*, 1994). Several studies demonstrated that in physiological conditions, an aromatic PU, such as Pellethane, could release their components into the body despite their apparent thorough design. The release can be caused by inflammatory cell activity (Maurin *et al.*, 1997; Zhang *et al.*, 1996), oxygenates, enzymes (Bouvier *et al.*, 1991; Meijs *et al.*, 1993; Santerre *et al.*, 1994), or even the sterilization procedure (Darby *et al.*, 1978). The released substances include PU native components and auxiliary agents that are added during the manufacture of the implants.

The demand for re-sterilization and reuse of electro-cardiac catheters has recently increased. Even though the Food and Drug Administration (FDA) recommendations are against the reuse of disposable cardiac catheters, which has incited manufacturers to label

electrode catheter packages as "single use", many institutions and hospitals continue to routinely re-sterilize and reuse them for cost-saving reasons. The problem that we are facing today is that there is unlimited re-sterilization and reuse of catheters in medical institutions without guidelines by which to operate. We know that repeated sterilization can lead to several adverse consequences in patients because of the risk of toxicity (Buben, *et al.*, 1999; Goldman *et al.*, 1997; Nair, 1995). Chemical residues that come from sterilization agents may remain on the catheter and be released into the circulation once implanted in the patient. In spite of this, there are very few studies concerning the potential cytotoxic risk related to multiple sterilization procedures (Conseil d'Évaluation des Technologies de la santé du Québec, 1994).

Macrophages are well known to be implicated in the host inflammatory and immune processes. Its response to biomaterials has a direct impact on the material biostability and biocompatibility. The adhesion of macrophages and the formation of foreign body giant cells containing fragments of PU materials have been observed on the surface of PU implants both *in vivo* and *in vitro* (Maurin *et al.*, 1997; Saad *et al.*, 1996, van de Lei *et al.*, 1987). The phagocytosis by macrophages of small PU fragments was also observed in surrounding tissues. Chronic inflammatory responses, accompanied by macrophage and/or foreign body giant cell accumulation, have been associated with various adverse complications, affecting both the host and the implant itself. Indeed, surface cracking was found to occur directly under these macrophages (Zhao *et al.*, 1990). Furthermore, activated macrophages also secrete cytokines, which are known to be implicated in one of

the mechanisms whereby cleavage of the PUs may occur. Even though many studies suggest the importance of macrophages in the biocompability of PUs, little is known about the effects of PUs in the attraction and activation of macrophage. We therefore applied an *in vitro* model designed by Catelas *et al.* (1998) in order to evaluate the macrophage response to PU. The present research project is divided into two parts: (1) Study of the potential cytotoxicity and activation effects of commercially available PU particles on macrophages; (2) Investigation of the potential cytotoxic effect of re-sterilized electrophysiology catheters on macrophages..

The first study on the interaction between PU particles and J774 macrophage showed that PU particles affected both the viability and the activity of J774 macrophages. The most important parameters that influenced macrophage response were particle concentration and their composition. The ratio of hard/soft segment may play a role in the attraction of macrophages. This study is presented in detail in the Materials, Methods, and Results sections of Chapter 5 (Article 1. Cytotoxic Reaction and TNF-α Response of Macrophages to Polyurethane Particles).

The results of second study show that re-sterilization of PU-based catheters has very low cytotoxic effect on J774 macrophages. The sterilization methods (steam autoclave, ethylene oxide or hydrogen peroxide plasma sterilization) and the number of sterilizations (up to 10 cycles) had no significant effect on the viability and TNF-α release of J774 macrophages. These findings suggest that if the functional characteristics

of catheters are not altered by sterilization, PU-based catheters can potentially be considered for reprocessing for up to ten cycles in hospitals. The Materials, Methods, and Results sections are presented in Chapter 6 (Article 2. Safety Issue of the Re-sterilization of Polyurethane Electrophysiology Catheters: An *in vitro* Cytotoxicity Study).

A general discussion based on our work is presented in Chapter 7. We also evaluated the validity of our methods and results, as well as bring forth future research directions. Our data on the re-sterilization of catheters may serve as a first step in the creation of safety norms for the reuse of catheters in hospital practices. The results demonstrating the potential cytotoxic effect of hard segment components contribute to the understanding of the interaction of PU materials with human environment. These results may also help direct research to develop safer biomaterials for future use.

CHAPTER 2. BIOMEDICAL POLYURETHANES

This chapter presents current knowledge on the biocompatibility of polyurethanes (PUs). We will first review the general information about PUs, including the history of their development, their chemical structure, as well as their medical applications. The focal point of this chapter is to present the factors that influence PU device performance in the human body.

2.1 History of Polyurethanes as biomaterials

Polyurethanes were introduced in 1937 by Otto Bayer of I.G. Farbenindustrie (Leverkusen, Germany) (Bayer *et al.*, 1947). The work led to the synthesis of novel materials via reactions between diamines and aliphatic diisocycayanates. PUs were introduced to the biomedical society in the 1960s and were used for the fabrication of different devices: (1) Diagnostic catheters (Cordis Corporation, Miami, FL); (2) Breast prosthesis (polyester urethane foam); (3) Cardiac lead insulation (Boretos *et al.*, 1967; Stoke *et al.*, 1986). Modifications of PU materials were based on their degradation behavior. Many efforts were put on producing "biostable" materials. The first patented, commercially available "biostable" PU material, named Corethane™ (Corvita Corp., Miami, FL, USA) was produced in the early 1980s. Other biostable PUs, such as Mitrathane™ (PolyMedica, Woburn, MA, USA), ChronoFlex® (Corvita Corp., Miami,

FL, USA) appeared in succession. The major products of the polyether urethanes are Pellethane® 2363 (Dow Chemical, La Porte, TX, USA) and Biomer™ (Ethicon, Somerville, NJ), which are widely employed in the manufacture of medical devices. Even today, attempts to modify polyurethanes are still pursued to optimize their properties (Anderson *et al.*, 1998). After three decades of improvements, PUs are considered as one of the most biocompatible types of biomaterials. They represent the ideal material for the making of various kinds of medical devices, especially for the repair and the replacement of soft or cardiovascular tissues (Szycher, 1991; Zdrahala, 1996).

2.2 Chemical Composition of Polyurethanes

PUs represent a large polymer family with different compositions and varied applications. These segmented copolymers are the reaction product of three different molecules, or monomers, which are generally referred to as the isocyanates (hard chain segments), the macroglycols (soft chain segments) and the chain extenders. Many different types of isocyanates, macroglycols and chain extenders are used in the plastic industry. The chemistry of PUs can vary according to the chemistry of the diisocyanate, the macroglycols, the chain extenders, as well as the auxiliary agents used for chemical reactions. Therefore, many combinations and permutations of these basic monomers yield PUs of vastly different chemical and physical properties in order to satisfy different demands. The most frequently used isocyanate for medical applications is the 4,4-methylene bisphenyl diisocyanate (MDI) which demonstrates good flexibility, high wet

tensile strength, and good thermal stability (Figure 2.1). The macroglysols include two classes: polyester and polyether. At the present time, polyether glycol is mostly used for medical applications and the most common one is polytetramethylene oxide (PTMO), which possesses great mechanical strength and water resistance (Figure 2.2). Chain extenders are used to extend the length of the hard segment and increase the hydrogen-bond density and the molecular weight of the PU (Figure 2.3).

Additives are added during the fabrication of commercially used polymers for the purpose of controlling and modifying the polymer reaction to facilitate manufacturing (Coury *et al.*, 1988). Synthetic anti-oxidants, such as Santowhite® and Irganox®, as well as vitamin E, have been added for the purpose of preventing the oxidation of PU soft segments (Anderson *et al.*, 1998).

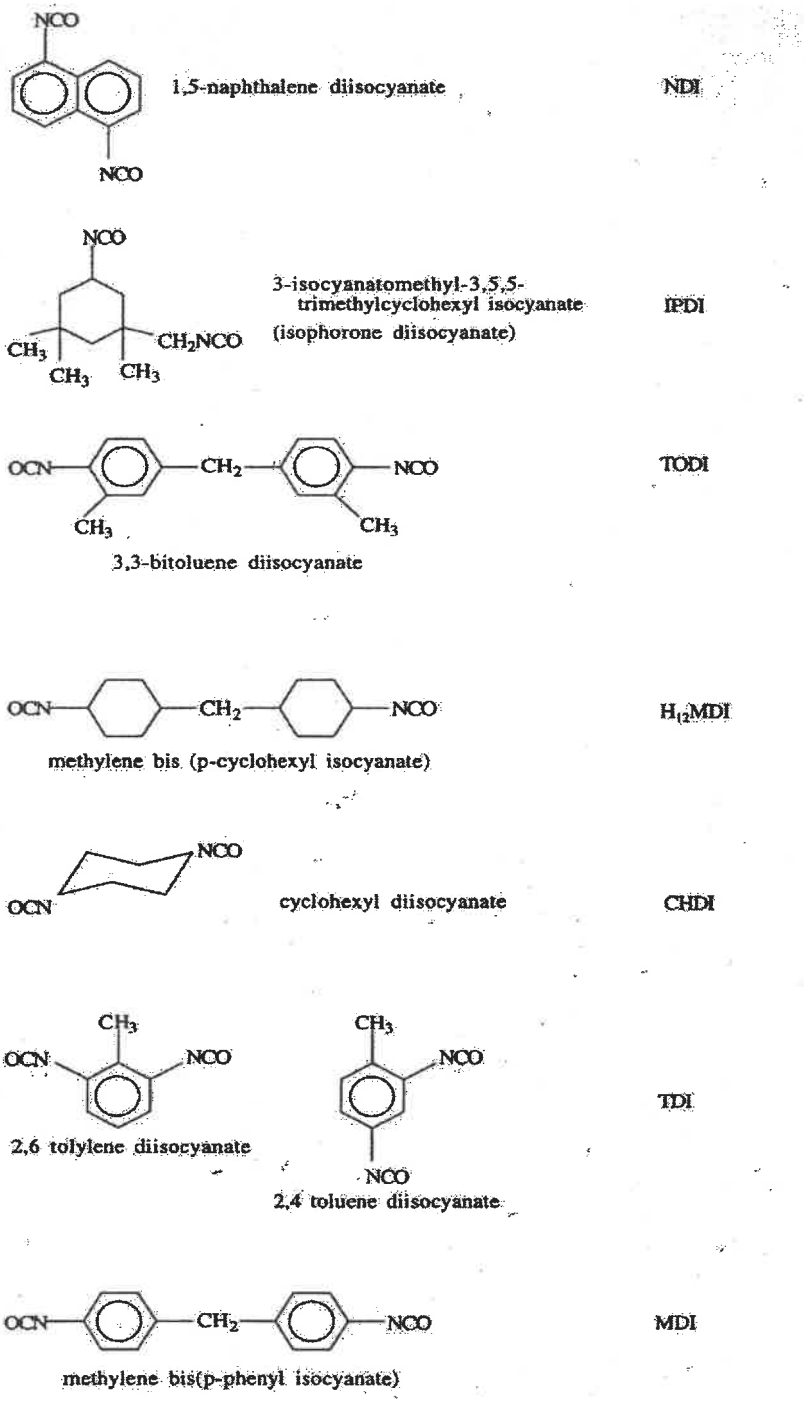


Figure 2.1. Chemical formula of some commonly used isocyanates in polyurethane synthesis. (Lamba *et al.*, 1998, *Polyurethanes in Biomedical Applications.* CRC Press. FL, U.S.A).

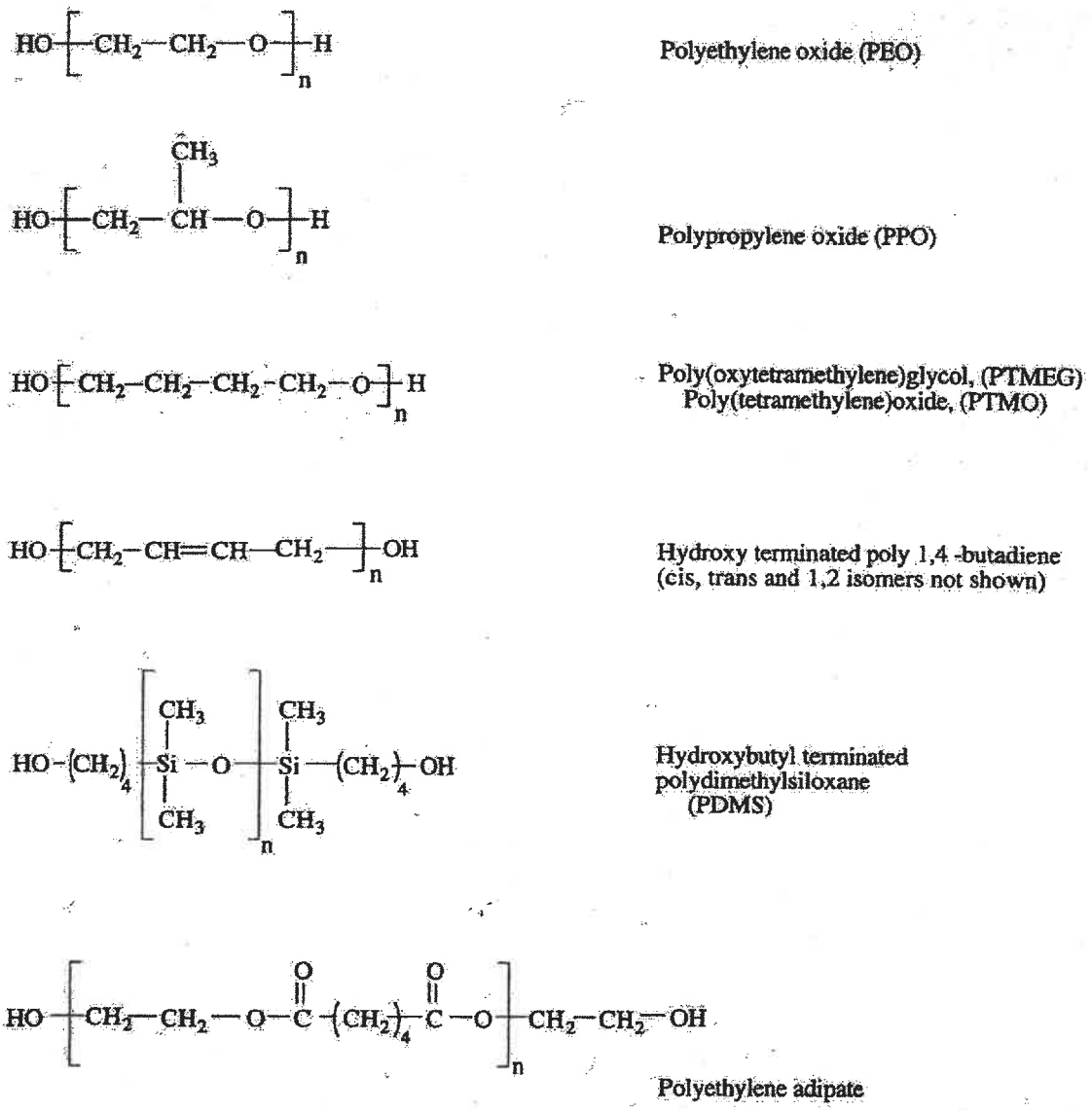
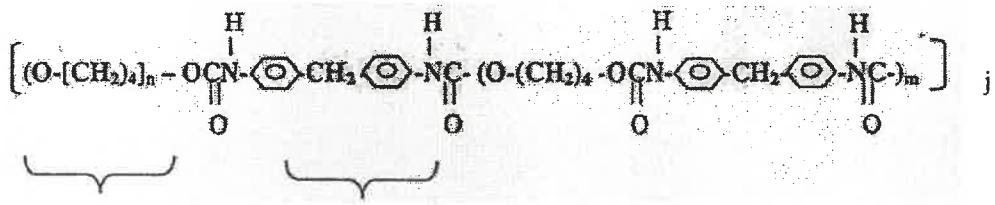


Figure 2.2. Chemical formula of some commonly used polyols in polyurethane synthesis. (Lamba *et al.*, 1998, *Polyurethanes in Biomedical Applications.* CRC Press. FL, USA).

$HO\text{-}CH_2\ CH_2\ CH_2\ CH_2\text{-}OH$	1,4 butanediol (BD)
$H_2N\text{-}\ CH_2\ CH_2\text{-}NH_2$	Ethylene diamine (ED)
$HO\ CH_2\ CH_2\text{-}OH$	Ethylene glycol (EG)
$HO\text{-}(CH_2)_6\text{-}OH$	Hexannediol (HD)

Figure 2.3. Commonly used chain extenders in the synthesis of polyurethane. (Lamba *et al.*, 1998, *Polyurethanes in Biomedical Applications.* CRC Press. FL, USA).

In the 1970s, a series of thermoplastic, segmented elastomers were developed by Upjohn Co. (New Haven, CT) and sold under the designation of Pellethane® 2363. These materials were synthesized from PTMO, MDI, and BD (Szycher, 1991; Lelah and Cooper, 1986). Tecothane® was also developed in the early 1970s (ThermoElectron, U.S.A) and is targeted at specific medical device applications, particularly for blood contact applications (Pinchuk, 1994). Pellethane® 2363 and Tecothane® are prepared with the same ingredients but in different ratios of hard/soft segments and additives. The basic molecular formula of Pellethane® and Tecothane® is shown in Figure 2.4.



PTMO ether soft segment MDI/BD hard segment

Figure 2.4. The basic chemical structure of Pellethane® and Tecothane®.

(McCarthy *et al.*, 1997, Biomaterials, 18, 1387-1409.)

2.3 Medical Applications of Polyurethanes

PUs have been chosen as biomaterials for various medical purposes because of their excellent properties: very high flexural endurance, good mechanical properties and relatively good blood compatibility. These advantages make them the ideal candidates for replacing soft tissues. Indeed, human connective tissue is elastic, tough, and compliant. Only two biomedical grade polymers approximate these characteristics - PUs and silicons. However, the mechanical properties of silicons are weaker than those of PUs. Therefore, no elastomeric materials can replace these tissues better than PUs (Szycher, 1991; Lamba *et al.*, 1998). PU devices range from catheters to vascular grafts, and from transient (diagnostic catheters, wound dressings) to permanent devices (pacemaker lead insulators, artificial organs) (Dumitriu *et al.*, 1994; Lamba *et al.*, 1998). They are increasingly accepted as one of the best types of biomaterials for the present and the future in a vast range of medical applications. Table 2.1 lists some of the PU applications to give a general picture of the standing of these excellent materials.

Table 2.1. Polyurethane in medical applications.

Application	Types of materials	Formulation
Bladders, chamber coating, catheter, Artificial heart	Biomer	MDI/PTMO/EDA/DAMCH/Soln
Cardiovascular application	Corethane	MDI/HMEC/BD
Vascular grafts	Cardiothane	MDI/PTMO/ BD
Cardiovascular, wound dressings, Vascular prostheses	Estane	Formulation varies
Vascular prostheses	Mitrathane	MDI/PTMO/EDA/Soln
Pacemaker lead insulation Blood bags, Catheter, Artificial heart	Pellethane	MDI/PTMO/ BD
Pacemaker lead insulation. Wound dressing	Tecoflex	HMDI/PTMO/BD
Blood contact applications	Tecothane	MDI/PTMO/ BD
Controlled drug delivery & other application	-	Formulation varies

MDI = methylene diisocyanate; PTMO = polytetramethylene diisocyante; HMDI = hydrogenated methylene diisocyanate; PDMS = poly(dimethyl siloxane); EDA = ethylene diamine; BD = 1,4-butanedial; DAMCH = diaminocyclohexane; HMEC = hexamethylene carbonate.

2.4 Biocompatibility

Biocompatibility has been defined as the ability of a material to perform with an appropriate host response in a specific application that does not result in any adverse tissue reactions (Williams, 1991). In other words, a biomaterial must act as similarly as possible to a natural tissue in its biological environment. Host response depends on many factors, including the chemical structure, the surface properties, the size, and the implantation site of the devices. It can also be influenced by various processes that are capable of altering the chemical structure of the materials, including manufacturing, sterilization, surgery, as well as degradation.

2.4.1 Breakdown of Polyurethane Devices in Biological Environments

The human body is a hostile environment for any foreign material, and polymers certainly suffer its influence after implantation. An important result of the contact with the physiological environment is polymer degradation that causes surface cracking. The earliest degradation of PU was observed in mammary implants made from polyester urethane foam (Herman *et al.,* 1984). Most of the scientific investigations on the degradation of polyether urethanes were done in the early of 1980s with the first report on a pacemaker lead insulator made from Pellethane® 2363-80A (Parins *et al.*, 1981). In some cases, surface cracking of pacemaker lead insulators progressed to the point of

breaching the insulator (Phillipes *et al.*, 1988). Later on, many studies showed PU degradation *in vivo* (Pinchuk, 1994; Stoke *et al.*, 1995; Szycher, 1985). Small diameter vascular grafts (diameter < 6 mm) are another type of interesting blood compatible devices. Unfortunately, these PU grafts failed due to degradation, with the appearance of cracks on the surface after one to six months of *in vivo* implantation (Martz *et al.*, 1988). Figure 2.5 shows the appearance of surface cracking of polyurethane material. Once micro-cracks were observed, sooner or later, significant failures occurred.

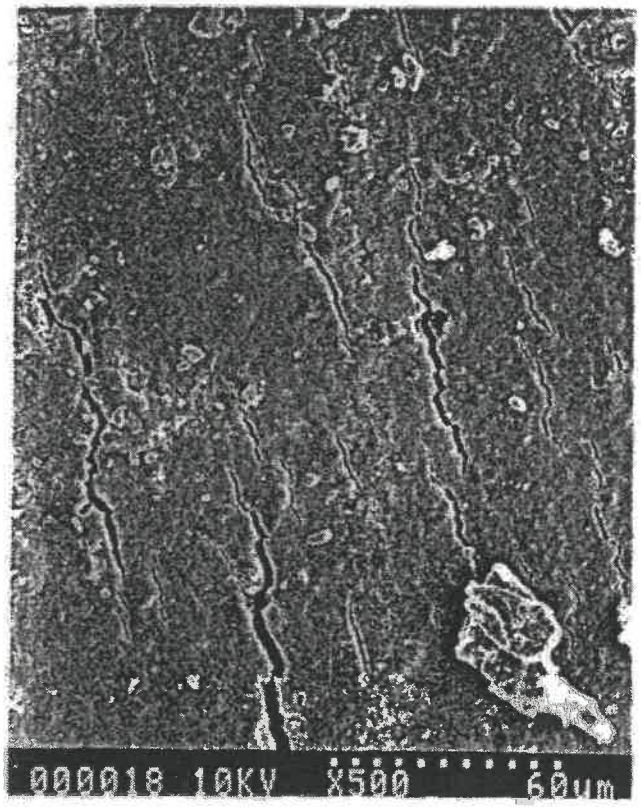


Figure 2.5. Surface cracking observed on polyurethane sheet.

(Meijs *et al.*, 1993, *J. Biomed. Mater. Res.*, 27, 345-356.).

Several investigations have shown that a certain amount of PU components are released from the implant and reach the material surface both *in vivo* and *in vitro* (Devor *et al.*, 1993; Wu *et al.*, 1991). Zhang *et al.* (1994, 1996) investigated PU-based vascular prostheses by observing their morphological, physical and chemical changes after implantation in humans. Their studies showed a prosthesis content loss on the external surface of the prostheses. They inferred that change was due to inflammatory cell activity. Meijs *et al.* (1993) reported that Pellethane® 2363-80A released its components after 24 h of treatment with hydrogen peroxide. Enzymes also caused Pellethane® 2363-80A release (Bouvier *et al.*, 1988). PU-based blood bags releasing of MDI was reported after steam autoclave sterilization (Darby *et al.*,1987). Presently, approximately 20 different substances released from PUs have been identified. They have been identified and classified into the following three classes:

(1) PU components: soft segments, hard segments, and chain extenders, including MDI, PTMO, and BD.
(2) Bioreaction products: 2,4-toluene diamine (TDA) and 4,4'-methylene dianiline (MDA).
(3) PU additive agents: anti-oxidants, silicone oils, stabilizers and other auxiliary agents used to facilitate large-scale synthesis and manufacturing.

The host response to these substances is different and may be attributed to a combination of polymer compounds and/or biodegradation products (van der Giessen *et al.*, 1996). All

PU components, including hard segment, soft segment, and chain extenders have potential toxic effects on the human body after the release from material. They may crumble into small pieces and be phagocytosed, eliciting or prolonging local inflammation: recognition by the host cells as a foreign body, phagocytosis by phagocytes, cell death and/or the secretion of inflammatory mediators, enhanced inflammatory response and prolonged material degradation.

The interaction of leachables or degradation products with tissues can change their surface properties and provoke a vast range of responses. These responses may then change the original characteristics of the biomaterial and even lead to prosthesis failure. The release or/and degradation components may also have cytotoxic effects, to the extent of causing carcinogenesis (Tsuchiya *et al.*, 1995, 1996). Furthermore, the released substances from the implant migrate to distant organs, particularly to the lymph nodes where accumulation of particle-containing macrophages may cause node enlargement leading to chronic lymphadenitis (Benz et al. 1996).

The influence of PU chemical structures on prosthesis biocompatibility has also been investigated both *in vivo* and *in vitro*. It has been reported that variations in the hard to soft segment ratio have significant effects on the biocompatibility of PU devices. Indeed, it is known that hard segment of Mitrathane, an aromatic polyether urethane used for the fabrication of vascular prostheses, is degraded and removed from the material surface. However, the biological effect of these components on surrounding tissues, their

interaction with host cells, especially with macrophages, and their role in the biodegradation and biostability of PU materials are still not clear (Paynter *et al.*, 1988; Batich *et al.*, 1989; Tsuchiya *et al.*, 1996).

2.4.2 Role of the Host in the Biocompatibility of PU-based Implants

Integration of an implant in any living tissue breaks the normal integrity of that tissue and, inevitably, a reaction to injury ensues. This response to a foreign material involves a cascade of events, the most important one being inflammation (Tang *et al.*, 1995). The inflammatory response is a dynamic, highly coordinated and regulated process, consisting of a network of interactions among a wide variety of cells and involving cell adhesion and secretion of more than 100 mediators (Beaman *et al.*, 1984). The characteristics of inflammatory responses are highly dependent on the inducing agent. However, one of the most important features of these inflammatory processes is debridement, the removal of dead and foreign materials (Aderem and Underhill, 1999).

Macrophages come from monocytes that are produced in the bone marrow and remain in the circulation during 12 to 32 days. From a physiological point of view, macrophages and multinucleated giant cells appear mostly in chronic inflammation. They migrate in response to chemotactic stimuli and provide a defense against invasion of the host by the phagocytosis of a wide variety of microorganisms, including bacteria, viruses, fungi, and protozoa. Phagocytosis is a dynamic process in which bacteria and/or foreign particles

are attached to the macrophage membrane in preparation for ingestion. This attachment step is mediated by specific macrophage receptors and depends on the nature of the microorganism or of the biomaterial. The macrophage is believed to be the primary component controlling this inflammatory response to biomaterials (Anderson *et al.*, 1988; Labow *et al.*, 1999; Rice *et al.*, 1998,). These macrophages are then involved in the biodegradation and biostability of polymers including PUs (Tseng et al., 1990; Tabata *et al.*, 1988; Vince *et al.*, 1991; Zhang *et al.*, 1996).

Over the past three decades, several investigations have been done on the factors involved in inflammatory responses to PU materials, including short-term and long-term healing processes. Results showed a chronic inflammatory response, accompanied by the accumulation of macrophages and/or foreign body giant cells around many types of PU implants (Morehead *et al.*, 1994; Zhang *et al.*, 1994, 1996; Zhao *et al.*, 1990). These inflammatory responses have been associated with various adverse complications, affecting both the host and the implant itself. An inflammatory response has also been observed surrounding PU-covered mammary implants and was associated with the degradation of the foam after 1-3 months of implantation in animals. This suggested that inflammatory responses play a significant role in the failure of PU materials (Picha *et al.*, 1990). Another study using scanning electron microscopy showed the presence of inflammatory cells around several kinds of polymers with a strong relationship with polymer surface degradation (Ali *et al.*, 1994). Finally, Mohanty *et al.* (1992) studied the soft tissue response to different PU prostheses implanted in different rat tissues.

Inflammation was observed on these prostheses and in the surrounding tissues after 15 days to 3 months of implantation.

The phagocytosis of fragments of foreign biomaterials are accompanied by the activation of macrophages and the initiation of polymer biodegradation (Ali *et al.*, 1994; Zhao *et al.*, 1990). These activated macrophages release free radicals and enzymes that affect oxidative chain cleavage. They also secrete a large number of pro-inflammation cytokines, notably tumor necrosis factor-α (TNF-α). TNF-α was originally characterized as a cytotoxic factor for many malignant cells. It is now clear that it plays an important role in the defense against viral, bacterial, parasitic infections, and in immune responses. TNF-α also modulates neutrophil functions (Hunt *et al.*, 1996). Finally, chronic inflammatory responses related to macrophage and/or foreign body giant cell accumulation around many types of PU implants are associated with various adverse complications, including hematomas and oxidative degradation of material (Marchant *et al.*, 1986; Casas *et al.*, 1999).

2.5 Significance of the Present Study

The above introduction and literature review allows one to conclude the following:

1) Although extensive studies have focused on the inflammatory response to PUs, few of them described the initiating and activating factors of macrophage action. The effect of PU composition on macrophage activation has not yet been reported.

Without the understanding of the interaction between macrophages and PUs, the stability of implanted PU biomaterials could not be predicted.

2) Most of the studies involved PU materials synthesized in research laboratories. In reality, PU biomaterials used today for the fabrication of medical devices are commercially available. These biomaterials contain several additive agents that may influence PU device biocompatibility.

CHAPTER 3: RE-STERILIZATION OF POLYURETHANE-BASED ELECTROPHYSIOLOGY CATHETERS

In this chapter, we introduce the history of the reuse of cardiac catheters and general information about PU sterilization. The advantages and disadvantages of sterilization methods and the safety issue of sterilization and re-sterilization of PU-based cardiac catheters will be discussed.

3.1 Background

Electrophysiology catheters are used for the mapping and the treatment of heart diseases. They improve and can even save patient lives. On the other hand, electrophysiology catheterization is expensive in terms of equipment. Electro-cardiac catheters were customarily reused before 1980. Policy against their reuse and the labeling of packages as "single use" was recommended by the American Food and Drug Administration (FDA) in 1977 based on the potential production of pyrogens in lumens of catheters. However, many hospitals and cardiac institutes continue to routinely re-sterilize and reuse electro-cardiac catheters because of increasing demands and economical considerations. For example, the University of Minnesota reused catheters from 1981 to 1986 (Dunnigan *et al.*, 1987). According to the report of the Conseil d'Évaluation des

Technologies de la Santé du Québec (1994), 31% of American hospitals reused cardiac catheters in 1986 (Institute for Health Policy Analysis, 1986), while this rate was 39% in Canada in 1991 (Canadian Coordinating Office for Health Technology Assessment, 1991).

The main reason for the reuse of catheters is the cost pressure on health care systems. Each catheter costs between $200 to $800 depending on the applications and the materials used. Each cycle of sterilization costs only $10 to $20. The financial benefit would then be significant in a repeated reuse of catheters. Avitall *et al.* (1993) estimated that reprocessing of 69 catheters in 336 ablation procedures (~ 5 reuses) would save up to $129,024. According to 1995 data, electrophysiology catheter costs are estimated at about $420 million in the United States (Lisa, 1995). If each reused catheter saves $160, a 50% reuse with 1.5 catheters per procedure can raise the savings to $50 million each year (Brown *et al.*, 1997). In Quebec, five uses for each diagnostic catheter and three uses for each angioplasty catheter would save about 6.5 million dollars each year.

In recent years, concerns for reuse practices increased due to the lack of data on the safety of reprocessing. Nevertheless, many hospitals still reuse catheters today. Thus, there is a great need for information concerning the safety issue of this practice.

3.2 Sterilization of Polyurethanes

Contamination and subsequent infection is the most important complication of the implantation of biomaterials into the human body. Some infections are fatal to patients, especially infections related to cardiac or vascular implants. Therefore, all medical devices must be sterilized to minimize the risk of infection.

Safety is a prime factor in the evaluation of sterilization methods. As for most polymer materials, PUs are sensitive to heat and moisture environments. Indeed, high temperatures cause chain scission and fragment release (Darby *et al.*, 1978; Jayabalan *et al.*, 1997). Therefore, methods with low temperature and low moisture are preferred to sterilize PUs. Currently used sterilization techniques for medical polymer devices include steam autoclave, ethylene oxide (EtO), irradiation, as well as low temperature gas plasma (Lamba *et al.*, 1998). The commercially available sterilization methods to sterilize PU materials are listed in Table 3.1. The advantages and disadvantage of these methods, usually depending on the composition of the device to be sterilized, will be discussed in the following pages.

Table 3. 1. Methods used for the sterilization of PUs

Method	Temperature	Time	Chemical Used
Steam	270°C 210°C	Flash: 4 min Dry: 20 min	–
EtO	20-60°C	15h	Ethylene oxide
Sterrad 100S (Gas Plasma)	<50°C	55 min	Hydrogen peroxide
Radiation	Room temperature	Long time	–

Steam. Sterilization by steam under pressure is the earliest and most traditional sterilization method applied to biomaterials. This method is carried out in an autoclave at a temperature higher than 121°C. The duration depends on the temperature, the configuration, and the packaging of the device. Steam sterilization is widely used because steam destroys most resistant bacterial spores in a brief period of exposure and the process is safer than the methods using chemical agents.

Ethylene oxide (EtO). EtO sterilization is an effective bactericide at low temperatures (between 20-60°C). EtO easily diffuses into materials and can sterilize heat- or moisture

sensitive materials. This is ideal for plastics such as PUs. Therefore, this method is presently the most largely utilized for the sterilization of polymers.

Radiation. This method uses the ionizing radiation, either of cobalt-60 (Co-60) or accelerated electrons. This method is convenient, effective, has negligible thermal effects, and does not involve toxic chemicals.

Sterrad® 100S. Sterilization technology based on gas plasma was patented in 1987 and marketed in the United States in 1993. Sterrad® 100S uses a diffusion stage with hydrogen peroxide (H_2O_2) followed by a plasma stage in each sterilization cycle (Rutala *et al.*, 1998). According to manufacturer this method is safe for the environment and healthcare workers, and does not leave toxic residues on biomaterials. It is simple to operate and no aeration is necessary. However, this method is quite new, thus we are not sure of its long-term effects on biomaterial stability.

3.3 Risks of toxicity related to the re-sterilization

Concerns about the safety of the re-use of catheters increased due to the fact that multiple uses and sterilizations of catheter may adversely affect their characteristics and functions negatively, not to mention that there is also a lack of data and regulations governing safest re-processing procedure. Nevertheless, many hospitals still re-use catheters today. Their re-use under these circumstances may result in the unsafe and ineffective treatment

of patients. Thus, there is a great need to understand the potentially toxic effects of sterilization on catheters in order to ensure the safety of this practice.

One of the greatest risks of re-sterilization is the potential toxicity that may be produced by the sterilization procedure. PUs are extremely sensitive to heated and moist environments. A high temperature will deform PU-based devices by hydrolysis or degradation. Indeed, medical grade PUs made from MDI are susceptible to degradation in aqueous solutions (Mazzu *et al.*, 1984). Therefore, steam autoclave is not a good method for the sterilization of PUs. In recent years, the attention on the biomedical field has increasingly focused itself on the risks generated by the chemical residues of EtO (Nair, 1995). Based on the data obtained from mice, the National Institute of Occupational Safety and Health has defined 800 parts per millions as the concentration that can cause damage to human life and health (National Institutes of Health, 1982). Polymeric materials can absorb ethylene oxide that possesses toxic properties even at low concentrations. Indeed, significant quantities of EtO represent significant health risks (Kudryk *et al.*, 1992; Weber and Rutala, 1998). Even though Sterrad 100S® seems to be a promising alternative to the EtO method, recent reports have shown that residues of the hydrogen peroxide used in the Sterrad 100S® may incite the degradation of a biomaterial through surface oxidation (Lerouge *et al.*, 2000) and induce a potential cytotoxic effect (Ikarashi *et al.*, 1995). After multiple sterilization cycles any sterilization method, which mentioned above, it is very possible that the catheter presents a threat to the health of a patient since both chemical residuals and degradation products can be released in the

patient's circulation during the use of a device. Therefore, the evaluation of toxic effects of catheters after repeated sterilization is of clinical relevance.

CHAPTER 4. MATERIALS AND METHODS

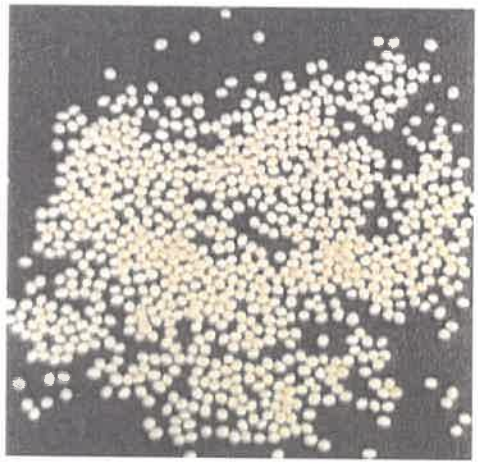
This chapter presents the materials and methods we used to study the effect of PUs on macrophages *in vitro*. These methods were chosen from (1) the review of the methods used by other researchers, (2) the availability of the experiment facilities and sample supply, and (3) the regulations of the American National Standard for the Biological Evaluation of medical devices (ISO 10993-5:1993).

4.1 Particles

4.1.1 Preparation of Particles

The commercially available Pellethane® series was used to prepare the particles (Figure 4.1). Pellethane® 2363 80 ABA40A (PL, Dow Chemical, La Porte, TX, USA), Tecothane TT2065DB40 (TC65; Thermedics, Wouburn, MA, USA), and Tecothane TT2085AB40 (TC85; Thermedics, Wouburn, MA, USA), were kindly provided by Caroline Guignot (Laboratoire des Polymers Irradiés, DSM/ DRECAM/ LSI, CEA Saclay, Gif-sur-Yvette, France).

(A)



(B)

(C)

Figure 4.1. Sample of Polyurethane pellets: (a) Tecothane® TT65DB40 (TC65); (b) Tecothane®TT85AB40 (TC85); (c) Pellethane 2363® 80ABA40 (PL).

We prepared our own particles due to the limitations in retrieving techniques and the putative alteration of debris during isolation, when using PU debris from patient tissues. PU extracts served as potential release products to assess the toxic effect of PUs under controlled conditions. The particles used were less than 5 μm, a size phagocytable by macrophages. PU particles were obtained by the modification of the solvent vapor method. Five mg of PL, TC65 and TC85 pellets were separately dissolved in 50 ml of tetrahydrofuran (THF) under stirring for 60 min. The PU-THF solution was then poured drop by drop into 500 ml of methanol (as non-solvent) under stirring. The gummy PU sediment was extracted from the suspension by filtration and spread into particles using an ultrasonic bath (Branson Sonefier 450, Danbury, CT, U.S.A) for 5 min or until particles completely separated. These particles were then filtered through a 5.0μm membrane (Fisher, Montreal, Canada) and then dried under reduced pressure. The particles were resuspended in methanol and kept at -4°C until use. Before each experiment, the particles were dried in air and sterilized by exposure to ultraviolet light for 24 h.

4.1.2 Analysis of PU Particle

Scanning Electron Microscopy Analysis

The shape, size, and size distribution of PU particles were analyzed through the use of scanning electron microscope (SEM) (Daka and chawla, 1993; Lanba *et al.*, 1998) . A Joel JSM-840A microscope (AEC Pretoria, Gauteng, England) was used at 15 kVolts.

Infra-Red Analysis

Infrared-Attenuated Total Reflectance spectroscopy (FTIR-ATR; Biorad-Excalibur Spectrometer, Edison, NJ, USA) was used for the identification of the chemical groups within the different particles (Guerra *et al.*, 1996; Vrmette *et al.*, 1999).

Gas Chromatography - Mass Spectroscopy Analysis

Gas Chromatography - Mass Spectroscopy (GC-MS; Agilent, Prochemist, Berkeley, USA) was also used for the analysis of particle composition (Weigand *et al.*, 1994).

Nuclear Magnetic Resonance Analysis

Nuclear Magnetic Resonance (NMR; ^{1}H-NMR-400MHz, Beuker, USA) was used for particle structure determination.

4.2 Sterilization of Catheters

4.2.1 Selection of Catheters

The catheters used in this study were new, single-use labeled, polyurethane-based electrophysiology catheters (Figure 4.2.). They are manufactured by Cordis Corporation (a division of Johnson & Johnson Medical Products, Florida, USA) and are widely used for diagnostic purposes. Cordis Corporation has been manufacturing cardiac catheters since the 1950s. They produce diagnostic cardiological catheters, which provide optimal "pushability" with outstanding torque response and minimal whip. These features allow the catheters to easily enter the vessels and cross the aortic valve causing minimal trauma to the patients.

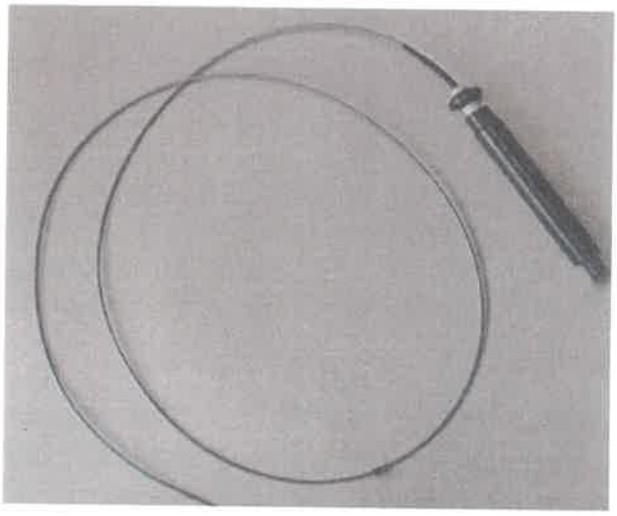


Figure 4.2. Sample of electrophysiology catheter

(Cordis Corporation, FL, USA).

4.2.2 Sterilization

Three sterilization processes were used: steam autoclave, EtO, and Sterrad 100S®. EtO and Sterrad 100S® are commonly used in hospitals and medical institutions to sterilize medical devices. They are also the most frequently studied methods of sterilization. Steam autoclave was used as a reference. Catheters were wrapped in self-sealing sterilization pouches and sent to the Sir Mortimer B. Davis - Jewish General Hospital (Steam autoclave and EtO) and Charle Lemoyne Hospital (Sterrad 100S®). They were then sterilized once or ten times under standard hospital conditions.

Steam autoclave

The flash technique was used for steam sterilization. Samples were autoclaved for 4 min at 270°C in a saturated humid atmosphere using a standard hospital autoclave. Catheters were then dried for 20 min. The catheters, which underwent ten cycles of sterilization, were treated ten respective cycles with the same procedure. No cleaning or washing exerted between the cycles.

Ethylene oxide (EtO)

EtO sterilization was performed in a Steri-Vac 3M system (London, ON, Canada) Catheters were exposed to a 60% humidity environment for 15 h at 55°C and aerated for 12 h. The catheters which underwent ten cycles of sterilization, were repeatedly exposed to the same conditions ten times. After sterilization, samples were degassed for two weeks.

Sterrad 100S®

The sterilization procedure of Sterrad-100S® is divided into two phases: at the diffusion phase, catheters were exposed to hydrogen peroxide at 50°C for 50 min. Then, catheters underwent a plasma phase that lasted 15 min. After the plasma cycle, samples were removed from the sterilization chamber to allow short-term chemical reactions to occur immediately after the process. The catheters, which had ten cycles of sterilization, were treated 10 times under the same conditions.

4.3 Cell Line

4.3.1 Selection of Cell Line

The J774 mouse macrophage cell line (ACTT, Rockville, MD, USA) was used in our *in vitro* model. Using a cell line allows us to avoid the influence of external sources and reduce the contamination potential, unlike samples from human blood peripheries. J774 macrophages have been successfully used to evaluate the cytotoxicity of several biomaterials. More specifically, they have the ability to phagocytose PU particles (Saad *et al.*, 1996; 1997) and other polymer particles (Holevinsky *et al.*, 1998).

4.3.2 Cell Culture

J774 macrophages were cultured in a RPMI 1640 culture medium (Biomedia Canada, Drummondville, Québec, Canada) supplemented with 5% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin at 37°C in a humidified atmosphere of 5% CO_2 in air. Culture flasks without catheters or particles served as negative controls.

4.4 Cytotoxicity Test

In our study, the cytotoxicity of re-sterilized catheters and PU particles on J774 macrophages was measured either through an indirect (catheters) or a direct (particles) method according to the American Association for the Advancement of Medical Instrumentation (AAMI, 1996). The viability of macrophages was determined by Trypan Blue exclusion and defined as the number of living cells divided by the total amount of cells. Cell mortality served as an *in vitro* marker of the cytotoxic effect of PUs on macrophages.

Indirect Contact

The indirect contact method was used to measure the cytotoxicity of catheters that underwent different sterilization methods. Samples were processed one or ten cycles with ethylene oxide, steam autoclave, and Sterrad 100-S® system under hospital conditions. They were then subjected to 72 h of extraction in a RPMI 1640 culture medium at 37 °C. The ratio between the surface area of the catheter and the extraction volume was fixed at 1.0 cm^2/mL. The extraction solution was used immediately after preparation. The medium of the unsterilized catheter extracts served as control. Macrophages (5 X 10^5/mL) were incubated with catheter extracts at 37°C for 24, 48 or 72 h.

Direct Contact

We used direct contact method to measure the cytotoxicity of PU particles on macrophages. Macrophages in suspension, at a density of 5×10^5 cells in 1 ml of culture medium, were exposed to PU particles at 10, 30, 50, 75, and 100 μg/ml. Culture tubes without particles served as negative controls.

4.5 Inflammatory Mediators

The concentration of TNF-α was measured by ELISA using a commercial kit (BioSource, Nivelles, Belgium). TNF-α served as an indicator of macrophage activity. This enzyme-linked immunosorbent assay is mouse specific with detection limits of 5 to 1250 pg/ml. The basic principle of this method lies in the use of antibodies conjugated to an enzyme which, by reacting with its subtract, forms a colored reaction product. The color intensity is measured with the help of a spectrophotometer and is directly proportional to the quantity of the dosed substance. At the end of each cytotoxicity test, culture media were collected and stored at –80°C. TNF-α was captured on a solid phase (matrix) with the resulting complex shown below (Figure 4.3). Consequently, this complex was incubated with the peroxyde subtract and the chromagene. The resultant color changes are directly proportional to the level of TNF-α. The absorbency was read at 450 nm.

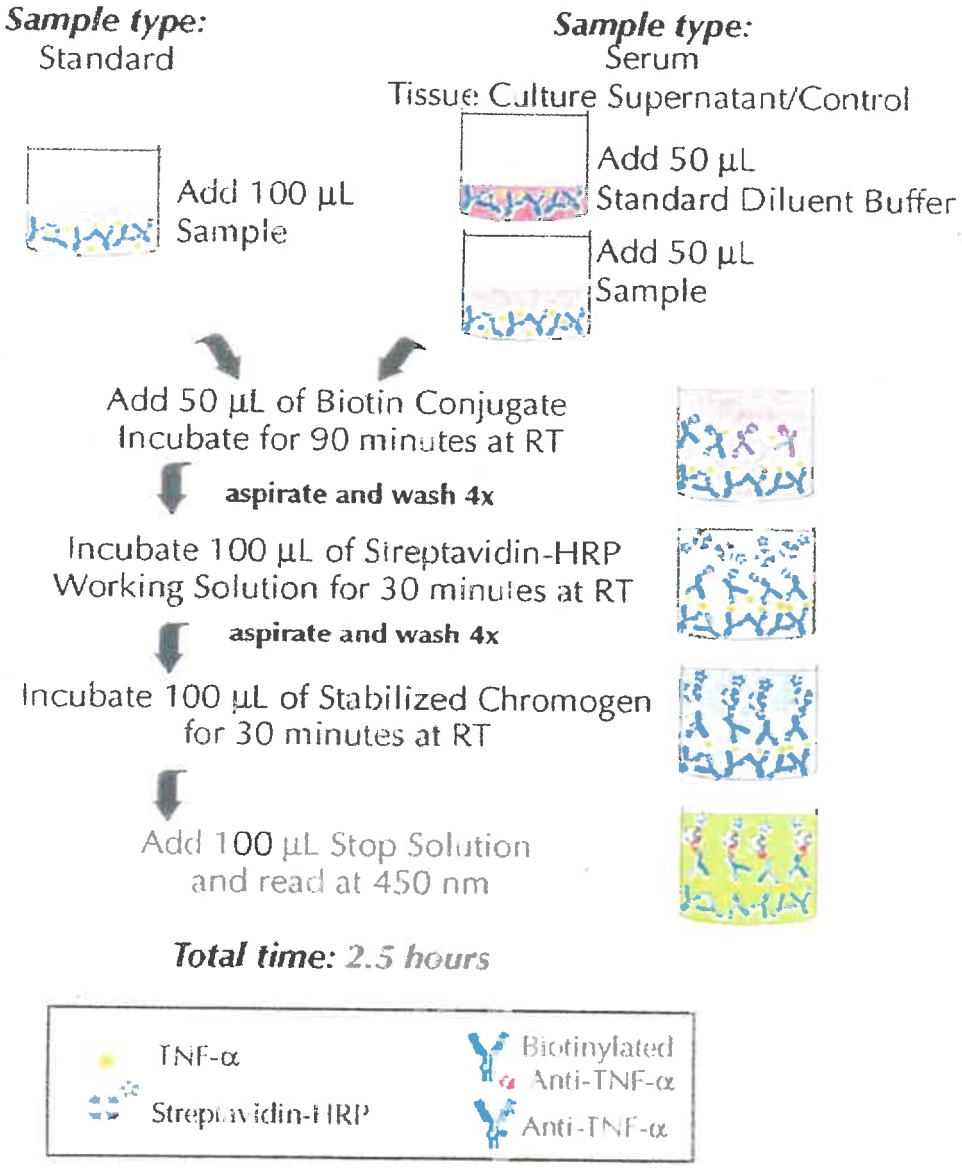


Figure 4.3. Illustration of the principle of the ELISA test

4.6 Statistics

Results were the mean of four experiments realized in duplicate. Statistical significance was evaluated by the analysis of variance (ANOVA). $P<0.05$ was considered statistically significant.

CHAPTER 5. CYTOTOXIC REACTION AND TNF-α RESPONSE OF MACROPHAGES TO POLYURETHANE PARTICLES

The literature review presented in Chapter 2 allows one to conclude that (1) Although extensive studies focused on inflammatory response to PUs, few of them described the initiating and activating factors of macrophage action. The effect of PU composition on macrophage activation has also not been reported. Without the understanding of the interaction between macrophages and PUs, the stability of implanted PU biomaterials cannot be predicted; (2) Most studies focus on PU materials synthesized in research laboratories. In reality, PU biomaterials used today for the fabrication of medical devices are commercially available. These biomaterials may thus be more representative of "true" host response.

The objective of our first section of study was to evaluate the macrophage response to the most commonly used polyether urethanes, namely Pellethane and Tecothane. Through this study, we looked at the cytotoxic effect of these PUs as a key event to determine the end of these materials in a physiological environment. These effects were measured by Trypan Blue exclusion, ELISA, as well as Western Blots. The results are presented in the article "Cytotoxic Reaction and TNF-α Response of Macrophages to Polyurethane

Particles" (Article 1), which was submitted for publication in the Journal of Biomaterials Science. Polymer Edition.

CYTOTOXIC REACTION AND TNF-α RESPONSE OF MACROPHAGES TO POLYURETHANE PARTICLES

Nan Ma[1], Alain Petit[3], L'Hocine Yahia[1], Olga L. Huk[3], *Maryam Tabrizian[2]

[1]GRBB, Biomedical Engineering Institute, École Polytechnique, Montréal, QC, Canada, H3C 3A7.

[2]Department of Biomedical Engineering, McGill University, 3775 University Street, Montreal, QC, Canada, H3A 2B4.

[3]Division of Orthopédic Surgery, McGill University, Lady Davis Institute for Medical Research, 3755 Chemin de la Côte Sainte-Catherine, Montreal, QC, Canada, H3T 1E2.

Short Title:

Macrophages Response to PU Particles

Correspondence:
Maryam Tabrizian, PhD, MBA
Department of Biomedical Engineering
McGill University
3775 University Street
Montreal, QC
Canada H3A 2B4
tabrizian@bmed.mcgill.ca

5.1 Abstract

Their unique mechanical and biological properties make polyurethanes (PUs) ideal materials for many implantable devices. However, uncertain long-term bio-stability in the human physiological environment limits their extensive clinical applications. Chronic inflammatory response associated with macrophage activation has been suggested as a prime factor; however, the mechanism of macrophage activation in response to biomaterial surfaces and debris is still unknown. The overall objective of this work was to study the response of macrophages to PU materials on *in vitro* by measuring cell viability and activity. This study was carried by using phagocytizable size PU particles from three types of commercially available polyurethanes: Pellethane® 2363 80ABA (PL), Tecothane® TT2065 (TC65), and Tecothane® TT2085 (TC85). These polymers possess the same generic composition but differ in additives as well as in the length of soft segment, as revealed by the FTIR and NMR studies. The results showed that polyurethane particles affected both viability and activity of J774 macrophages. The percentage of mortality ranged from 1 to 15% with 10 μg/ml to 100 μg/ml of particles after 24 h and 48 h incubation. These three types of particles induced different mortality on the macrophages. Specifically the mortality with PL particles was from 1 to 4%, while the mortality with TC85 particles was 2 to 10% and 4 to 15% with TC65. Conversely, these particles also affected cell proliferation. Cell numbers increased by 132% and 167% after 24h and 48h incubation respectively without particles, whereas the cell numbers reached only 46% and 78% of original cell number with TC65, 67% and

96% with TC85, and 66% and 107% with PL in the presence of 100 μg/ml of particles for the respective incubation times. PU particles also increased TNF-α release from macrophage. After have been incubated for 24h with 100 μg/ml particles of TC65, TC85, and PL, macrophages release TNF-α 7.4, 5.2, and 4.1 times as the control. In conclusion, PU particles had cytotoxic effects on J774 macrophage at high concentrations. The order of macrophage response to three type of particles was TC65 > TC85 > PL. The PU effect on macrophage activity depends not only on parameters already reported in the literature (i.e., size, shape, additive) but probably also on other variants related to the chemical composition and the ratio of hard to soft segments.

Key words: Polyurethane particles, Inflammatory response, Macrophage, Cytotoxicity

5.2 Introduction

For more than thirty years, polyurethanes (PUs) have been used as the choice biomaterial in many medical applications because they possess desirable mechanical properties and good biocompatibility [1,2]. The Pellethane® series was first introduced to the medical society in the 1970s. These polymers are Poly ether-based urethanes which are synthesized from 4,4-methylene bisphenyl diisocyanate (MDI), polytetramethylene oxide (PTMO), and 1,4-butanediol (BD) (Figure 5.1).

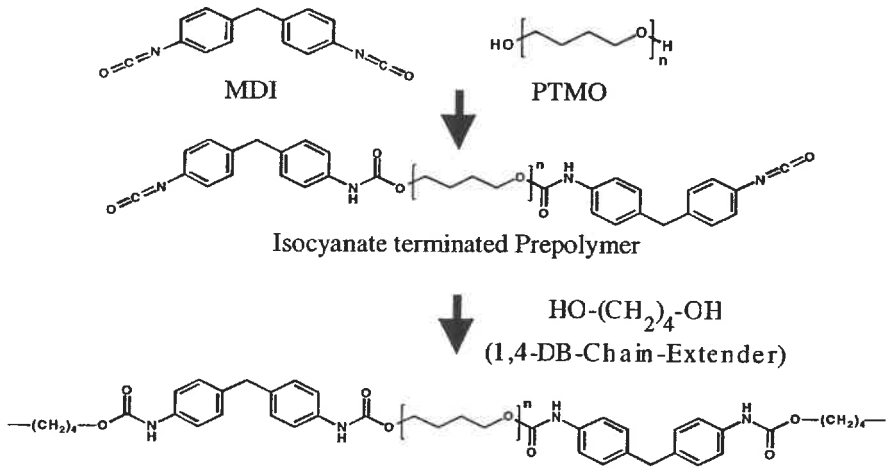


Figure 5.1. Synthesis of Pellethane® and Tecothane®

The Pellethane® series is used in heart pacemaker leads, blood bags, and cardiac catheters. It was also considered as a candidate in the fabrication of artificial valves and hearts [3]. However, potential applications are controversial due to the uncertain long-term effects of this material in the human physiological environment. The major cause of PU-based implant failure expressed in term of "surface cracking" [4, 5]. This phenomenon has been attributed to biochemical and cellular interactions at the surface of the implanted material that causes polymer chain cleavage as well as device malfunction and failure [6, 7].

Several studies have demonstrated that in physiological conditions, an aromatic polyurethane, such as Pellethane®, can leach its components into the body, whatever their apparent perfect design. Zhang *et al.* investigated PU-based vascular prostheses by observation of their morphological, physical and chemical changes after implantation in humans. Their study showed that the prostheses leaked contents onto the external surface of the prostheses. They suggested that this change was caused by inflammatory cell activity [8]. Meijs et al. reported that Pellethane® 2363-80A leaches its components after 24 h treatment with hydrogen peroxide [9]. Enzymes also cause low molecular weight species release from Pellethane® 2363-80A [10]. Degradation and/or particle release may be induced by sterilization procedures. Indeed, the release of small amounts of MDI from PU-based blood bags was observed after steam sterilization [11]. Both *in vivo* and *in vitro* investigations of PU implants showed strong evidence of leachable components to the surface [6, 12, 13]. In some cases, as many as 20 different substances have been identified

[14, 15]. These substances can be generally divided into two classes: (1) PU native components (soft segments, hard segments, and chain extenders), and (2) PU additive agents that are added during the manufacture, such as anti-oxidants, silicone oils, and stabilizers to facilitate large-scale synthesis and manufacturing.

All of the above substances are "foreign" to the human body. The mere presence of these leachables or/and degradation products on the surface layer may result in their being chemotacticly attracted to inflammatory cells. Their presence may also change the subsequent interactions between the PU-based implant and elements of the host environment. The biological effect of these release or degradation products and their interaction with surrounding tissues or host environment, especially with macrophages, as well as their role in the bio-degradation and bio-stability of PU materials are still not clear. It has been demonstrated that he implanted PU materials may cause local reactions (initial and prolonged inflammation, cancer development [16]) or systemic reactions (allergies[17]).

During inflammation, the monocytes are attracted from the blood vessels to the site of the foreign body. They mature into a macrophage after several weeks and accumulate on the surface of implanted material. The macrophages later fuse together to form foreign body giant cells [18]. Among the extensive studies that demonstrated a strong correlation between macrophage and PU biocompatibility [19, 20, 21], several indicated that fragments or particles from PU implants were small enough to be phagocytized by macrophages [22,

[23]. The interaction of phagocytic cells and implanted material affect PU stability. Indeed, it has been found that the surface of PU material cracks just under macrophage adhesion [24]. The adhesion of macrophages and the formation of foreign body giant cells which contain the fragments of breakdown material have been observed on the surfaces of PU-based implants, both *in vivo* and *in vitro* [25, 26, 27, 28]. The mechanism of macrophage activation in response to biomaterial surface is still unknown. However, it is known that macrophages play an important role in inflammatory response by their ability to release many types of cytokines that are implicated in this inflammatory response as well as in the recognition and clearance of apototic cells [29]. Among these cytokines, tumor nectosis factor-alpha (TNF-α) is known to initiate a variety of defense mechanisms and immunological responses [30]. TNF-α has been shown as an important cytokine produced in vitro by murine J774 macrophages in response to biomaterial particles [31]. Therefore, the aim of the present work is to investigate the macrophage response to PU particles to better understand the inflammatory response to PU leachables and/or degradation products.

Even though encouraging clinical results have been obtained, surface cracking remains the major obstacle for long-term PU applications. Furthermore, the study cell-PU interactions such as the toxic nature of PU native components and the effects of different additives are not known. Our investigation used polyurethanes with the same basic structure but different additives as well as different ratio of hard/soft segment in order to provide a new view of PU biocompatibility at the particles/cells level by measuring the

effect of PU on macrophage cytotoxicity and activity. The present work used commercially available PU materials, particularly the Pellethane® series [Pellethane® 2363 80ABA40 (PL), Tecothane® TT2065DB40 (TC65), and Tecothane® TT2085AB40 (TC85)], to simulate *in vitro* macrophages, that represent the predominant cell types involved in the foreign body reaction. By using a phagocytotable size of PU particles, we hypothesize that particles and/or degradation products release from a selected PU device may play an important role in the initiation of macrophage activation. We then focused in the present study on cell mortality as the indicator of cytotoxicity of PU particles and TNF-α release as an indicator of macrophage activity.

5.3 Materials and Methods

5.3.1 Polyurethane Particles

PU particles were obtained by the modification of solvent vapor method [32]. Five (5) mg of Pellethane 2363 80ABA40 (PL; Dow Chemical, La Porte, TX, USA), Tecothane TT2065DB40 (TC65;) and Tecothane TT2085AB40 (TC85; Thermedics, Wouburn, MA, USA) pellets were separately dissolved in 50 ml of tetrahydrofuran (THF) under stirring for 60 min. The PU-THF solution was then poured drop by drop into 500 ml methanol (as non-solvent) under stirring. The gummy PUs sediment was extracted from the suspension by filtration and spread into particles using an ultrasonic bath (Branson Sonefier 450, Danbury, CT, USA) for 5 minutes or until particles completely separated. These particles were then filtered through a 5.0 μm polycarbonate membrane (Fisher, Montreal, Canada) and then dried under reduced pressure. The particles were re-suspended in methanol and keep at -4°C until use. Before each experiment, particles were air-dried and sterilized by exposure to ultraviolet light for 24 h.

Scanning electron microscopy (JSM-840, JEOL, England) was used to determine the shape and the size distribution of the particles. Infrared-Attenuated Total Reflectance spectroscopy (FTIR-ATR, Biorad-Excalibur Spectrometer, NJ, USA), Nuclear Magnetic Resonance (^{1}H-NMR-400MHz, Bruker, USA) and Gas Chromatography-Mass

Spectroscopy (GC-MS, Agilent, USA) were employed to determine the chemical properties of particles.

5.3.2 Cell Culture

The J774 mouse macrophage cell line (ACTT, Rockville, MD, USA) was used as an *in vitro* model. The macrophages were cultured in RPMI 1640 medium (Biomedia Canada, Drummondville, Quebec, Canada) supplemented with 5% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5 % CO_2 in air. Cells in suspension were exposed to PU particles in tubes containing 5×10^5 cells/ml with particles at concentrations varying from 10 to 100 μg/ml. Culture tubes without particle served as negative control.

5.3.3 Cytotoxicity Test

Direct contact method was used for the cytotoxicity test according to the Association for the Advancement of Medical Instrumentation (ISO 10993-5:1993). Macrophages were incubated without or with PU particles for 24 h and 48 h at 37°C. Viability of the macrophages was defined by the number of living cells divided by the total of living plus dead cells using Trypan Blue exclusion.

5.3.4 TNF-α Release

The concentration of TNF- α was measured by ELISA using a commercial kit (BioSource, Nivelles, Belgium) and served as an indicator of macrophage activity. This assay is an enzyme-linked immunosorbent assay specific to mouse without cross-reaction with other cytokines. The detection limits of the assay ranged from 5 to 1250 pg/ml.

5.3.5 Statistical Analysis

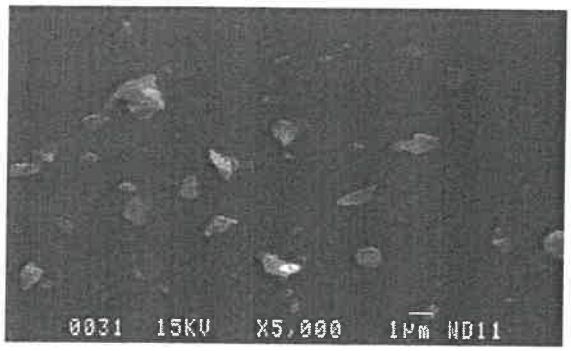
The results were reported by the mean of four experiments performed in duplicate. Statistical significance was evaluated by the analysis of variance (ANOVA). A probability level of less than 0.05 ($p < 0.05$) was considered statistically significant.

5.4 Results

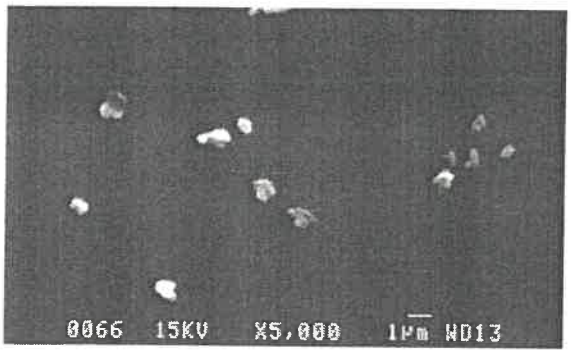
5.4.1 Physiochemical analyses of PU particles

Under scanning electronic microscopy, the shape of PU particles was irregular with a mixture of round, oval, and needle-shape (Figure 5.2). The size of particles ranged from 0.8 to 5.0 μm with a mean of 2.0 x 3.0 μm for PL, 1.0 x 2.5 μm for TC65, and 1.0 x 3.5 μm for TC85. From the FTIR-ATR, we observed absorption peaks at 1495 cm^{-1} that are characteristic of the hard segment (aromatic rings). Absorption peaks at 3300-3400 cm^{-1} are characteristic of the stretching of N-H bonds in isocyanate groups [33]. The FTIR-ATR spectra of all polyurethane samples behave with the same characteristics (Figure 5.3). The intensity of each peak is related to the concentration of the particular element.

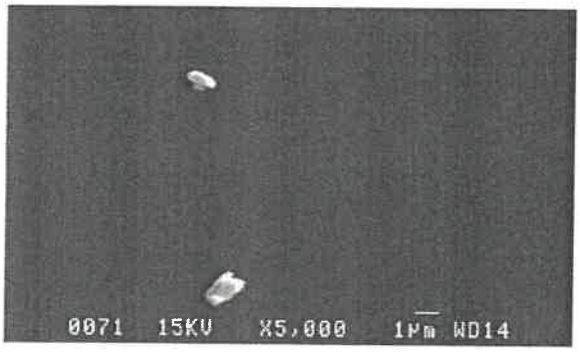
The ^{1}H-NMR analysis of polymers indicated again the similar compositions for PU samples (Figure 5.4). There was no trace of additives on spectra after the treatment of crude PU samples by organic solvents for the preparation of particles. The absence of additives in analyzed samples was also confirmed by GC-MS analysis. No peak of any small molecules corresponding to additives could be detected. Based on all of above analysis, the main components of these particles were similar to the original composition of PU pellets.



(A)



(B)



(C)

Figure 5.2. Shape of PU particles. PU particles were prepared as described in the section Materials and Methods and pictures taken by the author at a magnitude of ×5 000 on a scanning electron microscope. (A). Pellethane® 2363 ABA40; (B).Tecothane® TT2085AB40; (C). Tecothane® TT2065DB40.

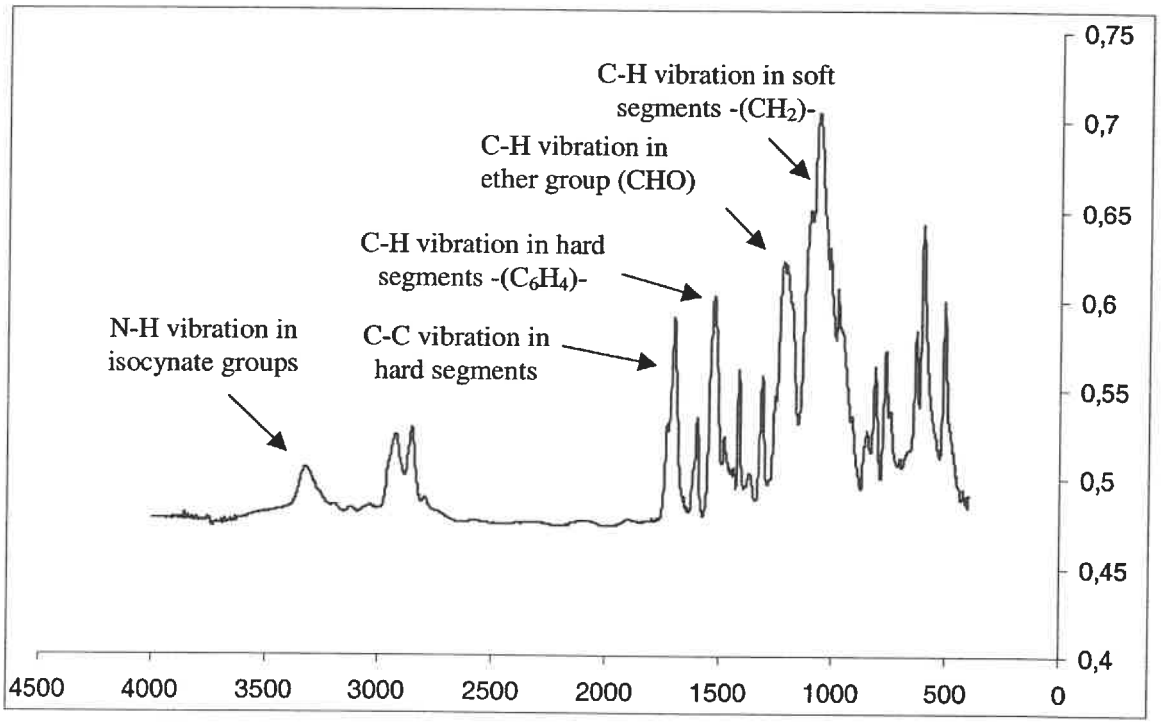


Figure 5.3. Fourier Transformed Infrared - Attenuated Total Reflectance (FTIR-ATR) spectrum of Pellethane®particles. PU particles were prepared as described in Materials and Methods and analyzed by FTIR-ATR spectroscopy (Biorad-Excalibur Spectrometer, NJ, USA).

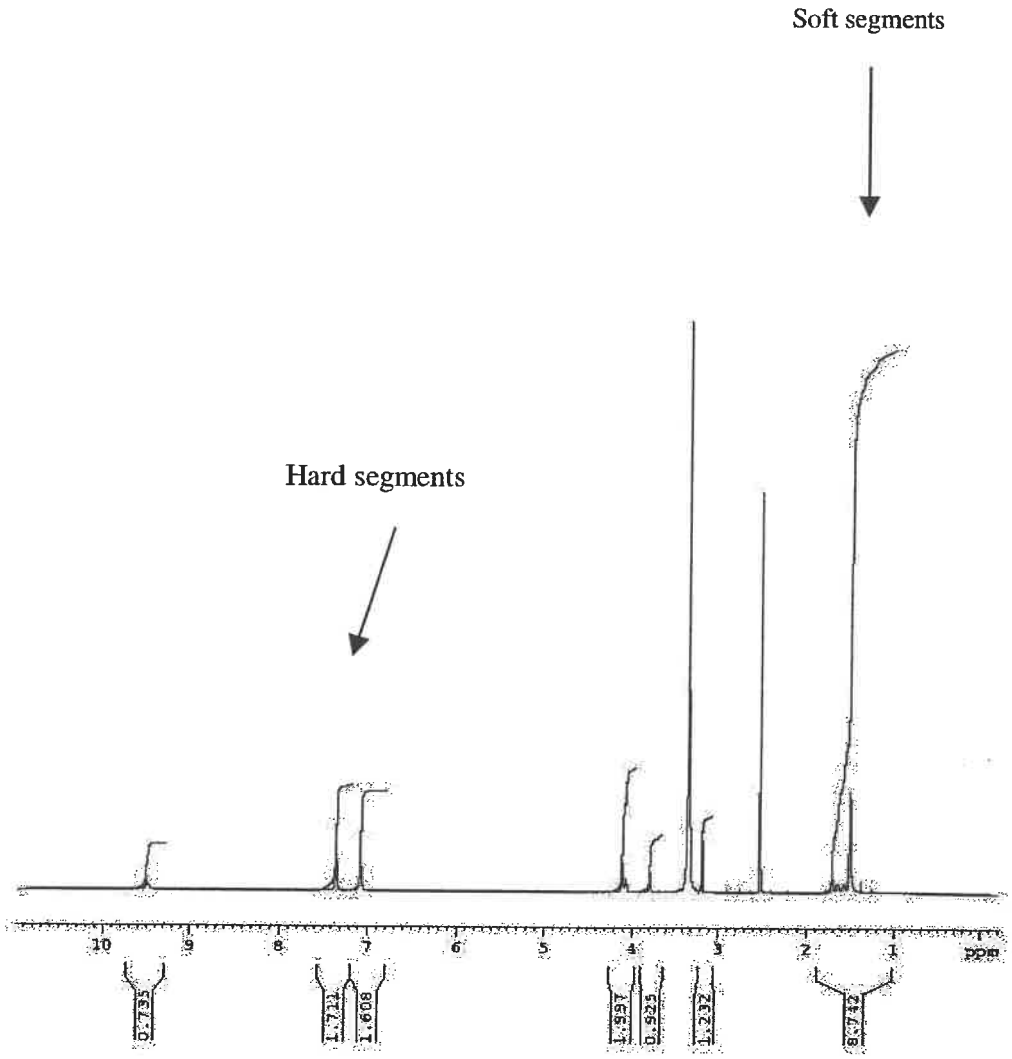


Figure 5.4. Nuclear Magnetic Resonance (NMR) analyses of Pellethane® 2363A80ABA particles. PU particles were prepared as described in the section Materials and Methods and analyzed by NMR (^{1}H-NMR-400MHz, Bruker, USA).

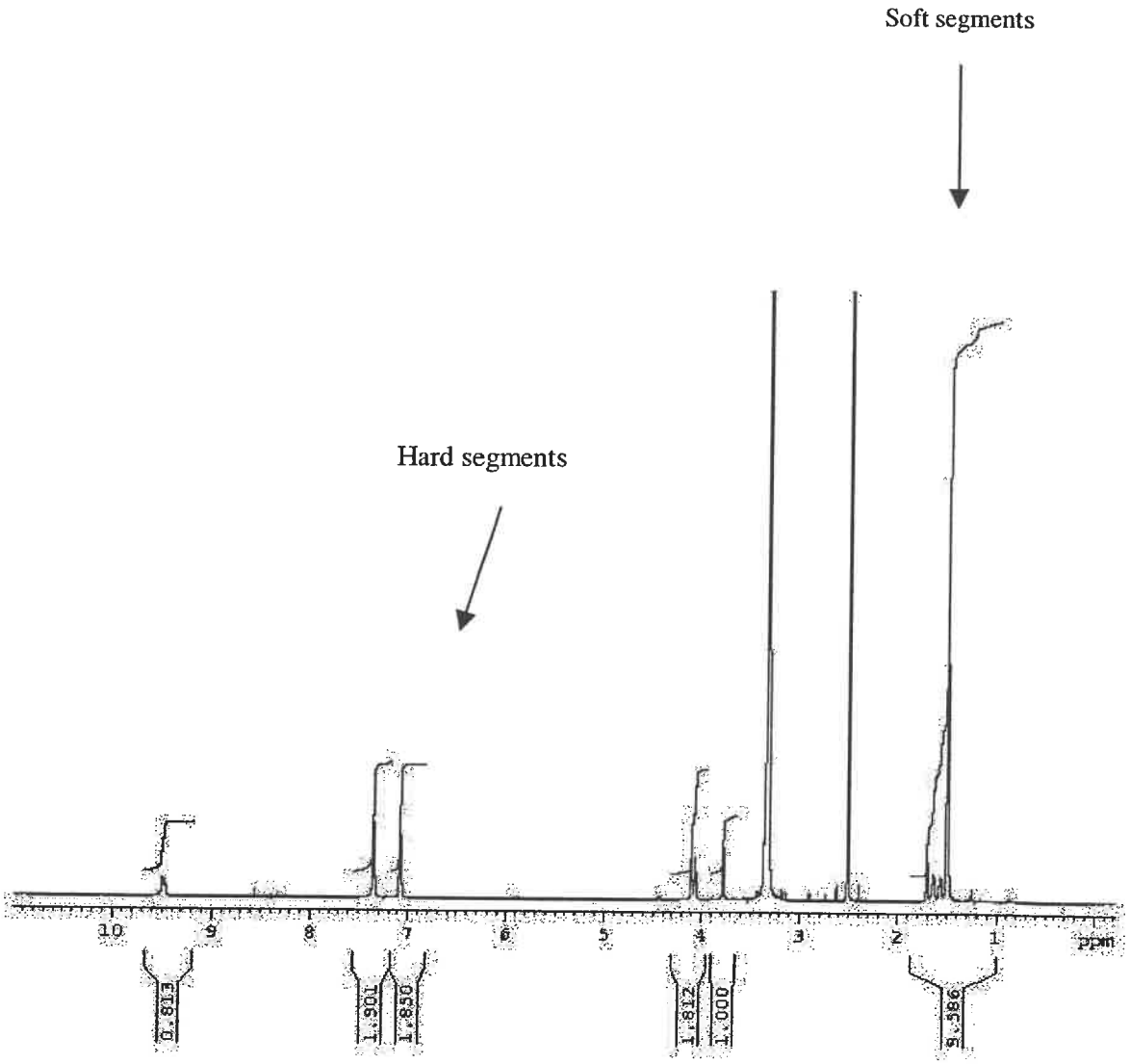


Figure 5.5. Nuclear Magnetic Resonance (NMR) analyses of Tecothane® TT2085AB40 particles. PU particles were prepared as described in the section Materials and Methods and analyzed by NMR (^{1}H-NMR-400MHz, Bruker, USA).

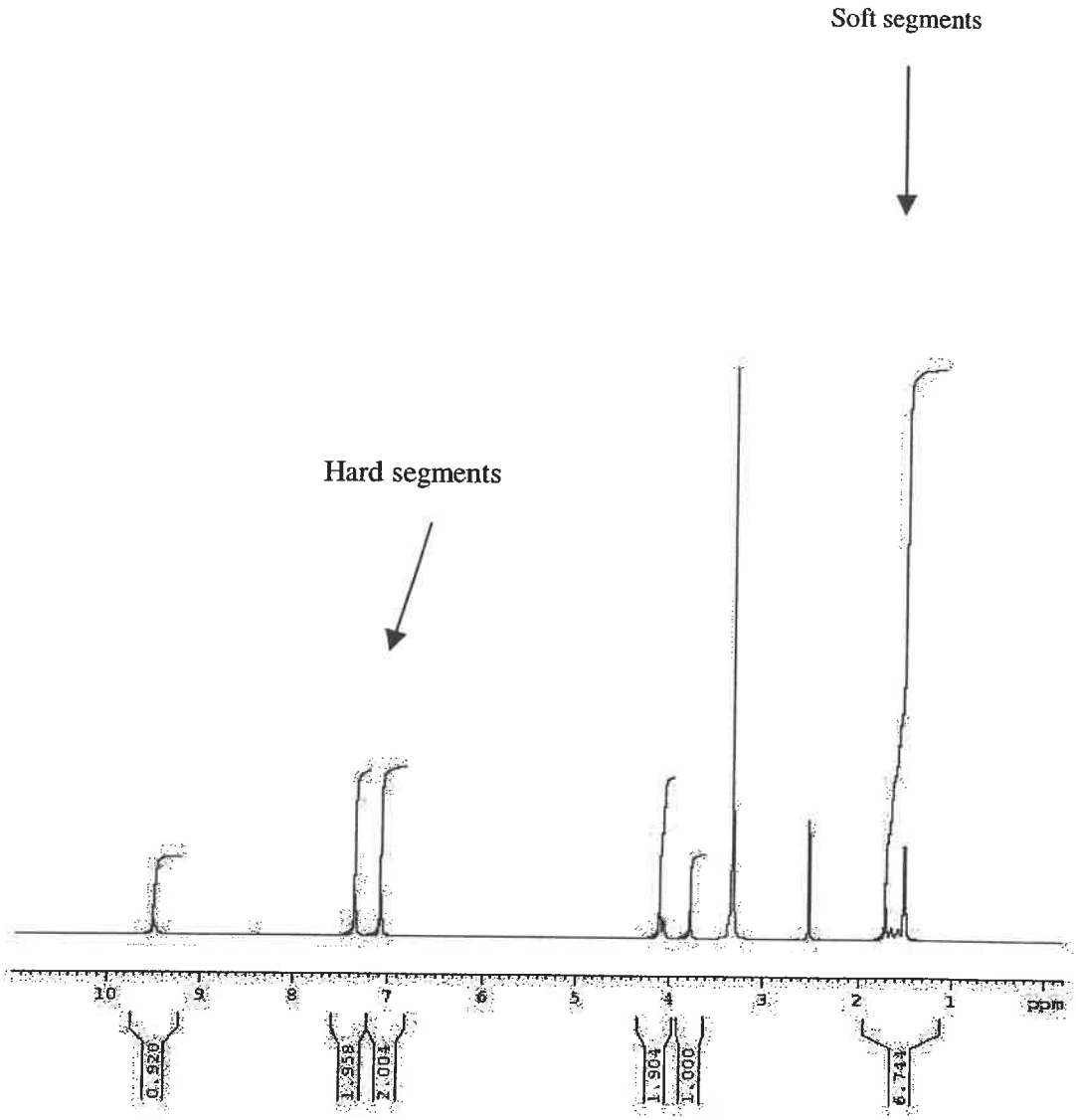
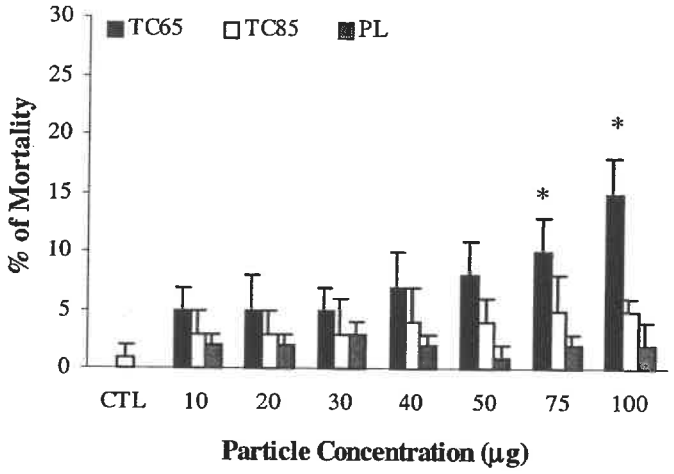


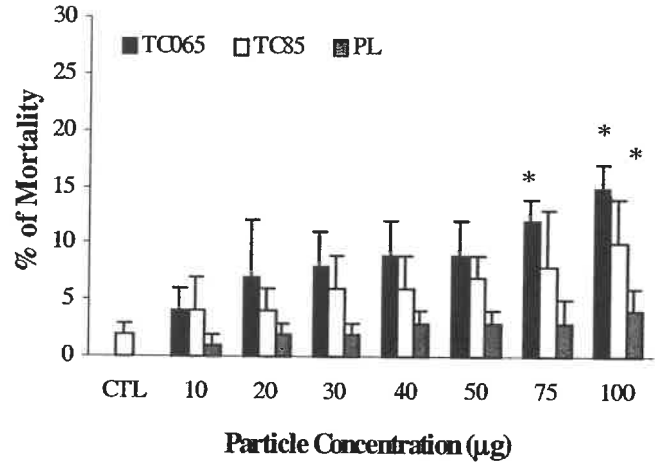
Figure 5.6. Nuclear Magnetic Resonance (NMR) analyses of Tecothane® TT2065DB40 particles. PU particles were prepared as described in the section Materials and Methods and analyzed by NMR (^{1}H-NMR-400MHz, Bruker, USA).

5.4.2 Biological Analyses of Polyurethane Particles

Figure 5.7 shows the effects of PU particles on the mortality of J774 macrophages. After 24 h incubation with 100 μg/ml of particles, the percentage of mortality reached 2%, 5%, and 15%, rising to 4%, 10%, and 15% with PL, TC85, and TC65, respectively, after 48 hours. There was no significant difference in cytotoxicity between PL and TC85 particles. However, the cell mortality was significantly higher ($p < 0.05$) in the presence of TC65. More specifically, PL showed the lowest mortality (4% after 48 h), TC85 showed a moderate mortality (10% after 48 h), while TC65 showed the highest one (15% after 48 h). In addition, there was no significant difference in cell mortality at 24h and 48h incubation time for any type of particles.



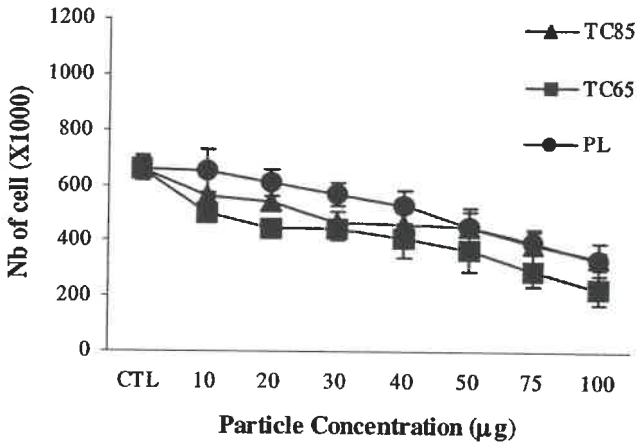
(a)



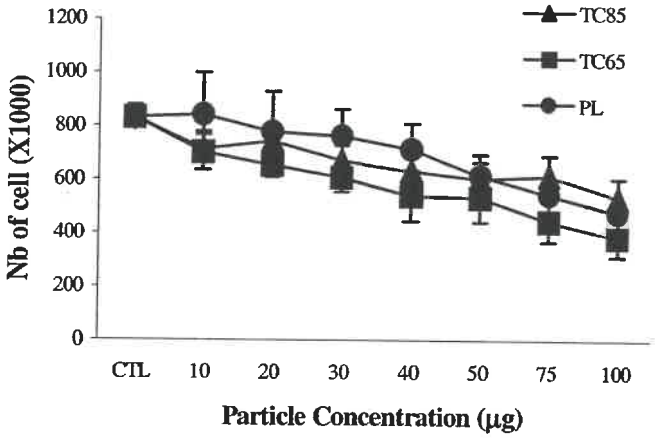
(b)

Figure 5.7. Effect of PU particles on macrophage mortality. J774 macrophages were incubated for 24 (a) and 48 h (b) at 37°C in absence (CTL) or presence of PU particles. Results are the mean ± standard deviation of 4 experiments performed in duplicate. *$p < 0.05$ vs. control.

In addition to analysis of cell viability, we observed significant differences in cell-growth rate with the different types of particles. (Figure 5.8.). The number of cells was lower in culture media in contact with PU particle samples compared with the controls (no particles). This phenomenon was particle concentration-dependent. After 24 h incubation, the cell number increased from 500 000/ml to 661 000/ml in control (33% increase). With 10 mg of particles, the cell population was 650 500/ml (30% increase), 562 000/ml (12% increase), and 501 500/ml (2% increase) with PL, TC85, and TC 65, respectively. The cell number decreases to 330 000/ml (34% decrease), 337 000/ml (33% decrease), and 232 500/ml (53% decrease) with 100 mg of PL, TC85, and TC65 particles, respectively. The cell number increased to 167% after 48 h incubation without particles whereas this number reached only 110, 107, and 77% of initial value with 100 mg of PL, TC85, and TC65 particles, respectively.



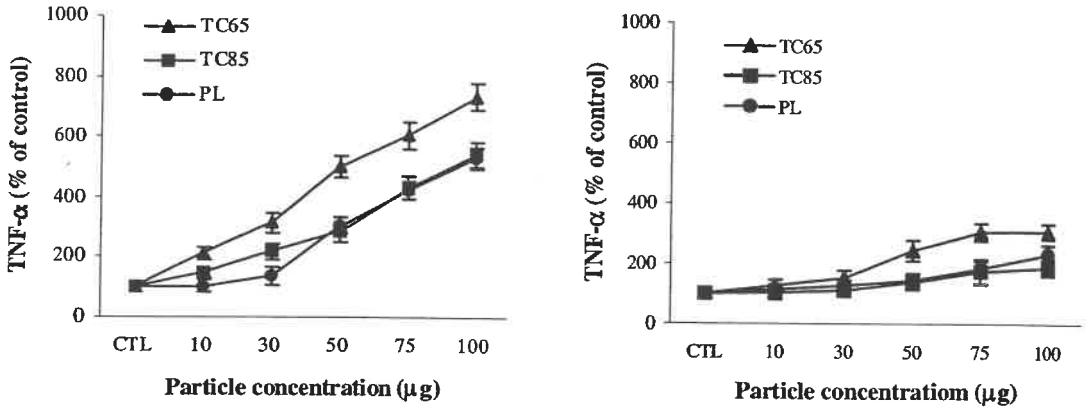
(a)



(b)

Figure 5.8. Effect of PU particles on the number of macrophages. J774 macrophages were incubated for 24 h (a) and 48 h (b) at 37°C in absence (CTL) or in presence of PU particles. Results are the mean ± standard deviation of 4 experiments.

Figure 5.9 shows the effect of PU particles on macrophage TNF-α release. We observed that the stimulation of TNF-α release reached a maximum at 24 h of incubation and decreased after 48 h incubation with PU particles. Again, the release of TNF-α was dependent on the type of PU: TC65 showed the highest effect on TNF-a release (758% of control after 24 h) while PL had the lowest one (529% of control after 24 h). TC85 stimulated TNF-α release by 541% after 24 h. We also observed a concentration-dependent effect of particles on TNF-α release for all analyzed particles. The results on TNF-α release were in agreement with those of cytotoxicity.



(a) (b)

Figure 5.9. Effect of PU particles on TNF-α release. J774 macrophages were incubated for 24 h (a) and 48 h (b) at 37°C in absence (CTL) or presence of PU particles. Results are the mean ± standard deviation of 4 experiments and are presented as % of control. Control = 165 pg/ml and 219 pg/ml after 24 and 48 h, respectively.

5.5 Discussion

The present study aimed at understanding the mechanisms that cause PU devices to be unstable for long-term application. We investigated commercial Pellethane® and Tecothane® series representing typical aromatic polyether-based PU used in clinical applications. Due to the limitation of retrieved tissue particles and the putative modification of the materials by extraction protocol, we used PU particles obtained by dissolving, precipitation and filtration from the above-mentioned medical grade PUs. As revealed from the FTIR-ATR and NRM spectra, all of these particles samples are with the same characteristics compared to the pellet samples. These particles might not exactly represent PU debris release in patient tissues in terms of size, shape and quantity, but they seem very appropriate for the *in vitro* study of the potential cytotoxicity of released PU components and/or biodegradation products. The macrophage is believed to be the primary component controlling inflammatory responses to polymer biomaterials [[26], [34], [35], [36]]. It plays a major role in the inflammatory response and wound repair process, and is the most common cell found on the surface and surrounding tissue of PU devices [[22], [37], [38]]. The present study used the particle model that might represent the state of fragmented polyurethane post degradation *in vivo*. The purpose was to investigate the potential cytotoxicity of released PU components on macrophages.

By Trypan blue exclusion, we showed that the cytotoxic effect of PU particles on J774 macrophages was dose- and type-dependent. We observed a significantly different

response of the J774 macrophage to three types of particles even when they had the same chemical structure. Our results are in contradiction with some previous studies showing no cytotoxicity with newly developed PU material [20, 21]. These results might be explained either by a difference between the chemical composition of our commercial PU and those synthesized by the authors, or by the difference in the shape, size, as well as by the presence of additives used during the manufacturing procedure of PUs. However, our chemical analyses did not show the presence of additives in our samples. Both experiments used phagotocytable size of particles. We then expect that the differential response of macrophages to PU particles originates from the difference in chemical composition (ratio hard/soft segments) and/or in the shape of particles used.

In addition to cell mortality, we observed a strong inhibitory effect of three polyurethane particles on J774 macrophage population after 24 h incubation. These effects were time- and dose-dependent, and also related to the type of particle. A significant inhibition effect could be detected with cells in contact with PU particles ($p<0.01$). This effect decreased after 48 h incubation and cell re-proliferation. This result may indicate that PU particles have an acute effect on J774 macrophage that is followed by a recovery phase. Our observation was very close to another PU stability study performed by Collier *et al.* Their explanation were related to the release of the antioxidant agent called dehydroepiandrosterone (DHEA) added in PU ether soft segments. The investigation showed that DHEA has a short time effect on inhibition macrophage adhesion since more than 70% of the DHEA had leached out of the samples within three days after

implantation. After that period, the concentration of macrophage adhesion on PU was the same both with and without DHEA [37]. An inhibitory effect of Pellethane leaching product has been also reported by Wu *et al.* Indeed, the Biomer™ surface pitting was a result of the leaching out of an additive named Santowhite powder, an antioxidant shown to inhibit the formation of foreign body giant cells and to inhibit surface cracking [39]. However, in the absence of additive in our PU samples, the cytotoxicity effect of particles cannot be related to the anti-oxidizing or any other chemical agents. Conversely, it is known that the biodegradation PUs appears only after interaction with host phagocytic cells on the material surface and that long-term inflammatory response involves long-lived foreign body giant cell [24]. The possibility of degradation of polymer chain is then very low in our experiments. It is thus very difficult to consider that some substances released from Pellethane® may limit the proliferation of macrophages within 24 h to 48 h and create such a source of chronic inflammatory response.

The explanation of the difference in macrophage response to the three types of PU particles is also very complex. All particles had the same chemical composition, the same shape and size, and they were free of additives. Our hypothesis lies on the results obtained by NMR analyses whereas a difference can be detected in the ratio of hard/soft segments of the three different particles. Based on the integration of various protons in ^{1}H-NMR spectra, the ratio of hard/soft segments varies from 1/7 to 1/4 for PL and TC65 respectively. That strongly suggests that the hard segments may be more toxic than the soft segments. Some authors already reported on the effect of polymer hard segments on

cell reaction [40, 41, 42, 43]. Toxic effect of PU mammary implant has been examined by Fourier Transform Infrared (FT-IR) and Gas Chromatography-Mass Spectrometry (GC/MS). Fragments of aromatic structures, specially toluene diamine (TDA), have been detected on the PU implant surface. The authors concluded that the contact of these fragments with phagocytic cells at the implantation site results in late pain and other allergic reactions to this implant. An other study evaluated Pellethane surfaces using Scanning Electronic Microscope (SEM). They observed an increase of macrophage adherence, cytoplasmic spreading, and macrophage-macrophage membrane fusion to form foreign body giant cells in function with the increase of Pellethane hardness.

Cytokine secretion is an indicator of macrophage activation and is a controlled key event in the initiation resolution and repair processes of inflammation. TNF-α was originally characterized as a cytotoxic factor for many malignant cells. It is now clear that it plays an important role in the defense against viral, bacterial and parasitic infections, and in immune responses. Our results show that J774 macrophage increased TNF- α release in contact with PUs particles. The concentration of TNF- α release reached a peak in the first 24 h incubation and reduced after 48 h incubation. TNF- α release increases with the increasing PU particle concentration although the numbers of cells decreased. These results suggest that our PU particles have the ability to inhibit the proliferation of macrophage, while, in the same time, increase their activity. A similar increase of TNF- α release by contact with PU material *in vivo* has been previously reported [44]. The increasing TNF- α level may augment the phagocytosis activity of macrophages and

cytotoxic effect of PU particles, regulating inflammation and wound repair at the site of implantation. Therefore, it appears that during inflammation, PU breakdown products cause the activation macrophages that secrete cytokines, the latter stimulating again macrophages, prolonging inflammation as well as inducing further degradation. We suppose that chronic inflammatory response is greatly depend on long duration of PU chemical component release. Based on the results of our study, we assume that the leaching or/ and releasing of substances from PU material *in vivo* may cause deterioration of their properties. Over time, they may exert a toxic effect into human environment and influence PU device stability.

5.6 Outlook

Investigation on the interaction of PUs with macrophages provided very useful information on PU biocompatibility in order to understand the mechanism of prosthesis failure and to improve their biological properties. This study compares the cytotoxicity of three types PU using a particle model. The results demonstrated that PU particles not only induced cellular mortality and inhibited cell proliferation, but also activated macrophage TNF-α release. The number of cells did not correlate with the amount of TNF-α produced from macrophage. More importantly, the results suggest that the ratio of hard segment/soft segment might play a key role in the induction of macrophage response to these particles. We suppose that the overall balance that decides a PU implant fate after implantation may depend on the concentration of PU breakdown and/or leachable products, the composition of released substances, and the level of cytokines. However, further investigations on cell-PU interaction are necessary to determine the cell response as a function of hard segment length.

Acknowledgement

This work has been supported by an NSERC operating grant. The authors thank C. Guignot for providing polyurethane samples, and S. Polizu and B. Thierry for their help in the electronic scanning microscope and infrared spectroscopy analyses.

5.7 References

[1]. Lamba NMK, Woodhouse KA, Cooper SL,: Polyurethanes In Biomedical Application, CRC Press LLC, Boca Raton, FL. 1998.

[2]. R.J. Zdrahala, *J. Biomater. Appl.* 1, 37 (1996).

[3]. M. Szycher, *High performance biomaterials*. Technomic Publishing Inc. p. 233 (1991).

[4]. K. Stokes, R. McVenes, and J.M Anderson, *J. Biomater Appl.* 9, 321 (1995).

[5]. M. Jayabalan, *J. Biomaterials Appl.* 8, 64, (1993).

[6]. M. Szycher, A.M. Reed, and A.A. Siciliano, *J. Biomater Appl.* 6, 110 (1991).

[7]. S.R. Labow, J.D. Erfle, and P. J. Santerre, *Biomaterials.*; 16, 51 (1995).

[8]. Z. Zhang, Y. Marois, R.G. Guidoin, P. Bull, M. Marois, T. How, G. Laroche, and M.W. King, *Biomaterials.* 18, 113 (1997).

[9]. G.F. Meijs, S.J.McCarthy, E. Rizzardo, Y.C. Chen, and R.C. Chaterlier, *J. Biomed Mater Res.* 27, 345 (1993).

[10]. M. Bouvier, A.S. Chawla, and I. Hinberg, *J. Biomed Mater Res.* 25, 773 (1991).

[11]. T.D. Darby, H.J. Johnson, and S.J. Northup, *Toxicol Appl Pharmacol.* 46, 449 (1978).

[12]. O.H. Zhao, J.M. Anderson, A. Hiltner, G.A. Lodoen, and C.R. Payet, J. *Biomed Mater Res.* 26, 1019 (1992).

[13]. R. Phillips, M. Frey, and R.O. Martin, *Pacing Clin Electrophysiol* 9, 1166 (1988).

[14]. T.M. Sinclair, C.L. Kerrigan, and J. Sampalis, *Plast Reconstr Surg.* 96, 1326 (1995).

[15]. G.B. Wang, J.P. Santerre, and R.S. Labow, *J. Chromatogr B Biomed Sci Appl.*, 26, 69 (1997).

[16]. R.A.F. Clark and P.M. Henson, in: *The molecular and cellular biology of wound repair.* Plenum Press, New York (1988).

[17]. J. Pauluhn, *Arch Toxicol.* 74, 257 (2000).

[18]. G.J. Bellingan, H. Caldwell, S.E.M. Howie, I, Dransfield, and C. Haslett, *J. Immunology.* 157, 2577 (1996).

[19]. H.M. Luu, J. Biles, and K.D. White, *J. of Applied Biomaterials*; 5, 1 (1994).

[20]. R.S. Labow, E. Meek, and J.P. Santerre, *J. Biomater Appl.* 13, 187 (1999).

[21]. L. Tang, and J.W. Eaton, *Am J Clin Pathol.* 103,466 (1995).

.[22]. N. Maurin, N. Daty, C. Guernier, K. Dahmen, and H. Richter, *J Biomed Mater Res.* 34, 73 (1997).

[23]. B. Saad, S. Matter, G. Ciardelli, G.K. Uhlschmid, M. Welti, P. Neuenschwander, and U.W. Suter, *J Biomed Mater Res.* 32, 355 (1996).

[24]. Q. Zhao, M.P Agger, M. Fitzpatrick, J.M Anderson, A. Hiltner, K. Stokes, and P. Urbanski, *J Biomed Mater Res.* 24, 621 (1990).

[25]. M. Szycher and W.A. McArthur, in: *Corrosion and Degradation of Implant Materials*, p. 308, Fraker and Griffin, eds. Philadelphia (1985).

[26]. F.M. Benoit, *J Biomed Mater Res.*27, 1341 (1993).

[27]. D.E. Devor, M.P. Waalkes, P. Goering, and S. Rehm, *Toxicol Pathol.* 21, 261 (1993).

[28]. T. Tsuchiya, R. Nakaoka, H. Degawa, and A. Nakamura, *J Biomed Mater Res.*31, 299 (1996).

[29]. A. Aderem and D.M. Underhill, *Immunol.* 17, 593 (1999).

[30]. D. Mooney, M. O'Reilly, and R.L. Gamelli, *Ann Surg.* 211, 124 (1990).

[31]. C. Catelas, O. Huk, A. Petit, D.J. Zukor, R. Marchand, and L'H. Yahia, *J Biomed Mater Res.* 41, 600 (1998).

[32]. M. Sato, T.F. Xi, A. Naksmura, Y. Kawasaki, T. Umemure, M. Tsuda, and Y. Kurokawa, *J Biomed Mater Res.* 29, 1201 (1995).

[33]. T.G. Grasel and S.L. Cooper, *Biomaterials.* 7, 315 (1986).

[34]. J.M. Anderson, and K.M. Miller, *Biomaterials.* 5, 5 (1984).

[35]. J.M. Rice, A.C. Fisher, and J.A. Hunt, *J. Biomater. Sci. Polymer Edn.* 9, 833 (1998).

[36]. D.F. Williams, in: Biocompatibility, an overview. In the Concise Encyclopedia of Medical and Dental Matarials. Williams DF Pergamon Press, Oxford, UK (1991).

[37]. T. Collier, J. Tan, S. Matthew, M. Shive, S. Hasan, A. Hiltner, and J. Andersin, *J Biomed Mater Res.* 41, 192 (1998).

[38]. R. Guidoin, M. Therrien, C.Rolland, M.King, J.L. Grandmaison, S.Kaliaguine, P.Blais, H. Pakdel, and C. Roy, *Ann Plast Surg.* 28, 342 (1992).

[39]. Y. Wu, Q. Zhao, J.M. Anderson, A. Hiltner, G.A. Lodoen, and C.R. Payet, *J Biomed Mater Res.* 25, 1415 (1991).

[40]. Casas, Q. Zhao, M. Donovan, P. Schroeder, K. Stokes, and D. Untereker, *J. Biomed Mater Res.* 15, 475 (1999).

[41]. T. Tsuchiya, R. Nakaoka, H. Degawa, and A. Nakamura, *J. Biomed Mater Res.* 31, 299 (1997).

[42]. C. Batich, J. Williams, and R. King, *J Biomed Mater Res*, 23, 311 (1989).

[43]. M. R. Brunstedt, J. M. Anderson, K. L. Spilizewski, R. E. Marchant, and A. Hiltner, *Biomaterials*, 11, 370 (1990).

[44]. J. A. Hunt, B.F. Flanagan, P.J. McLaughlin, I. Strickland, and D.F. Williams, *J Biomed Mater Res.* 31, 139 (1996).

CHAPTER 6. SAFETY ISSUE OF THE RE-STERILIZATION OF POLYURETHANE ELECTRO-PHYSIOLOGICAL CATHETERS: AN *IN VITRO* CYTOTOXICITY STUDY

Chapter 3 demonstrates the importance of the measurement of potential toxic effects of sterilization and re-sterilization on electrophysiology catheters. After multiple-cycle sterilization, the catheters may present toxic risks to the patients due to the presence of chemical residues from the sterilization process, and of toxic polymer degradation products released on the surface of the catheters as a result of sterilization. Both chemical residues and degradation products can be released into the patient's circulation after implantation. Therefore, the evaluation of the toxic effects of resterilized catheters is of clinical relevance.

The objective of our second study was to investigate the cytotoxicity of re-sterilized polyurethane-based electrophysiology catheters on macrophages *in vitro*. The viability of cells was measured by Trypan Blue exclusion while the activity of the cells was measured by ELISA. The results of this study entitled "Safety Issue of the Re-sterilization of Polyurethane Electrophysiology Catheters: An *in vitro* Cytotoxicity Study

(Article 2) has been submitted to the Journal of Biomedical Materials Research. Applied Biomaterials for publication.

SAFETY ISSUE OF THE RE-STERILIZATION OF POLYURETHANE ELECTROPHYSIOLOGY CATHETERS: AN *IN VITRO* CYTOTOXICITY STUDY

Nan Ma[1], *Maryam Tabrizian[1], Alain Petit[2], Olga L. Huk[2], L'Hocine Yahia[1]

[1]Group GRBB. Biomedical Engineering Institute, École Polytechnique, Montréal, QC, Canada, H3C 3A7.

[2] Division of Orthopedic Surgery, McGill University, Lady Davis Institute for Medical Research, 3755 Ch. Cote Ste-Catherine, Montreal, QC, Canada, H3T 1E2.

Correspondence:

*Maryam Tabrizian, PhD, MBA

Biomedical Engineering Institute
Biomaterial/Biomechanics Research Group
Ecole Polytechnique of Montreal
Montreal, Quebec
Canada, H3C 3A7

tabrizia@grbb.polymtl.ca

6.1 Abstract

The reuse of electrophysiology catheters may potentially result in significant cost savings. However, re-sterilization of catheters could lead to several adverse consequences, including toxicity related to potential chemical reactions that occur during sterilization of catheters since toxic residues may remain on the surface of catheters. The objective of this study was to investigate *in vitro* the cytotoxicity of polyurethane (PU)-based catheters on macrophages after their re-sterilization. We have compared three sterilization methods: steam autoclave, ethylene oxide, and hydrogen peroxide plasma. Our results showed that sterilization of PU-based catheters has very low cytotoxic effect on J774 macrophages. The viability of cells varied from 90% to 99% without any correlation with extraction or incubation times. Sterilization method (steam autoclave, ethylene oxide or hydrogen peroxide plasma sterilization) and the number of sterilization (up to 10 cycles) had no significant effect on the viability of J774 macrophages. Finally, extracts of PU-based catheters had no effect on TNF-α release by J774 macrophage. These findings suggest that if the functional characteristics of the catheter do not change, PU-based catheters can be potentially considered for repossessing up to ten cycles in hospitals.

Key words: Catheter, Re-sterilization, Cytotoxicity, Polyurethane, Safety

6.2 Introduction

Cardiovascular catheters are routinely used in clinical diagnosis and treatment. They play an important role in modern cardiology surgery. Since late 1970's recommendations against reuse of disposable cardiac catheters, most of the catheters available in the market are intended for single use. However, due to the significant cost of catheters and to the amount of medical waste generated by the use of disposable devices, the need arises to reuse them after proper reprocessing.[1] Reusing cardiac catheter is a worldwide practice. There are some reports on the success of multiple uses and sterilization cardiac catheters.[2, 3] However, it is difficult to collect data on how many cardiac catheters were reused each year in the world and how many reprocessing were performed for each catheter. According to the report of the Canadian Coordinating Office for Health Technology Assessment, 39% of hospitals in Canada reused cardiac catheters in 1991.[4] The potential cost savings of reusing catheter have been estimated. Each standard electrophysiology catheter costs from $200 to $800, and one cycle of sterilization costs only $10-$20. The average of five reuses of diagnostic catheters or three reuses of angioplasty catheter would save $1000–$4000 per catheter.[1] When catheters were reused an average of five times, a significant cost savings reached $129.024 over 336 ablation procedures.[5]

The catheterization is an intravascular procedure where the catheter will contact with patient's blood. Infection risk is then very high. Therefore, catheters must be sterilized strictly between uses in order to assure safety of the patients. Even though reused catheter can results significant cost saving, the safety of sterilization is always been concerned when the catheter are reprocessed because currently practiced sterilization methods have shown some adverse effects on biomaterial. The ideal re-sterilization procedure for cardiac catheters should satisfied the following requirements: a) destruction (or kill) of all microorganisms that may be present on the catheters in case of infection risk; b) maintain of catheter physio-chemical characteristics in order to assure safe and effective for use; c) and be free of toxicity risks for the patients.

Medical-grade polyurethanes (PUs) are a family of elastomeric materials whose chains are composed of alternating low glass transition (soft) segments and more rigid polar urethane (hard) segments.[6] PUs have excellent mechanical properties, such as high tear strength, high level flexibility, and resistance to abrasion. They also possess good blood and tissue compatibility, which have made PUs a popular choice of material for the construction of cardiovascular implants and devices including cardiac catheters.[7,8] However, it is well known that PUs are sensitive to sterilization processed, which may not only alter their physicochemical stability, but also may induce a toxicological risk.[9] The potential toxic effect is associated with PU physico-chemical alterations after their sterilization, such as degradation or surface modification. Those changes may cause PU chain scission and leaching of fragments as well as toxic agents. The second toxic

possibility is related to chemical residuals which potentially formed during sterilization and that may remain on catheters and would be harmful to patients.[10]

Degradation of polymer materials during sterilization has been noticed. The major underlying causes of PU degradation are hydrolysis and oxidation. The potential toxic and carcinogenic nature of releasing compounds were reported.[11] All of the components of PUs, the soft segment, hard segment, as well as chain extenders, are potentially toxic. Aromatic isocynates, such as 2,4-toluene diamine (TDA) may cause asthma[12] and contact dermatitis[13]. PUs with 4,4'-diphenymetbane diisocyanate (MDI) are susceptible to degrade if sterilization procedure is performed in the presence of moisture and if autoclaved in an aqueous solution.[14] Steam autoclave is then not recommended for PU sterilization.

On the other hand, PUs degradation cause by sterilization is more often associated with oxidative processed than with hydrolysis. There are several different mechanisms involved in these oxidative processes. Studying the effect of plasma-based sterilization on surface and bulk properties, and on hydrolytic stability of catheters, we recently showed surface modifications on all types of catheters.[15,16] An increased release of oligomers was also observed after sterilization. The changes in the surface and physical properties of PU catheters may then result in changes in material biocompatibility.

Currently used sterilization techniques for medical polymer medical devices include steam autoclave, ethylene oxide, and the hydrogen peroxide plasma method. Each sterilization has its advantages and disadvantages, usually dependent upon the composition of the device to be sterilized.[17]

Steam sterilization by autoclaving has traditionally been the universal method for medical instruments. The sterilization is achieved by exposing materials to saturated steam at 121°C. This requires a pressurized chamber and the process usually lasts between 15 min and 3 h. For immediate use, there is flash sterilization that needs a minimum of 10 min at 270°C in a gravity-displacement sterilizer (or 4 min at 270°C on a prevacuum sterilizer). Steam sterilization destroys most resistant bacterial spores in a brief exposure. It is also simple and lacks toxicity.[18] Since the last three decades, the number of synthetic polymeric materials used for medical application increased considerable. For example, Polytetrafluoreethylene (PTFE) has be used in vascular grafts; Polyvinyl chloride (PVC) is used in tubing for biomedical applications such as blood transfusion, feeding, and dialysis; PUs are used in pacemaker lead insulation, vascular grafts, heart assist balloon pumps, and artificial heart bladders.[19] Most of polymeric materials are heat/or moist sensitive. Steam sterilization may changes polymers' surface morphology and may have significant effect on biocompatibility or blood contact properties of the materials.[20] Therefore, this method is recommended for heat and moisture-stable biomaterial, such as metal or ceramic.

Ethylene Oxide (EtO) has been used because it is a simple method and is an effective bactericide at temperatures as low as 60°C. Since EtO can easily diffuse into materials to be sterilized, it is possible to sterilize heat- or moisture-sensitive materials. EtO is therefore ideal for plastics and has been considered the best method for polymers.[18] However, the main disadvantages of EtO sterilization is the residual of toxic agent, which may left in the material after sterilization. This issue becomes more critical for re-sterilizing polymeric devices because the porous nature of these materials. EtO is a reactive biocide moderately toxic for human. It directly alkylates proteins and deoxynucleic acid and is mutagenic in mammalian cells *in vivo* and *in vitro*.[21] Human exposure mainly occurs through inhalation in sterilization facilities and from sterilized medical equipment. EtO is irritating for eyes, skin, and respiratory tract. It can also cause life-threatening pulmonary disease, sensory and motor neurological impairment, cardiovascular collapse, or renal failure .[21] In the literature several studies reported a high level of residual EtO on re-sterilized catheters.[22] The concentrations of EtO were up to eight times greater than the maximal levels of the Food and Drug Administration's limit (< 25 ppm) for implantable products.[22]

Since few years, a new low temperature sterilization technique, so called hydrogen peroxide gas plasma, has been introduced. The process utilizes radiofrequency energy and hydrogen peroxide vapor to create a low-temperature hydrogen peroxide gas plasma and achieve relatively rapid sterilization.[22] The process temperature is kept at less than 55°C. It is a promising alternative to EtO for reprocessing of electrophysiology catheters,

efficient, free of the potential hazards of toxic chemical residuals, and easy of maintenance. Finally, it does not require aeration time as for EtO.[23, 24] However, H_2O_2 has been recognized as a germicide for more than a hundred years. It showed strong cytotoxicity.[18] H_2O_2 residual remaining in the materials is potentially harmful for human. The oxidative activity and cytotoxicity of materials sterilized with hydrogen peroxide has been also reported.[25] H_2O_2 in the PU materials is however eliminated after 12h aeration. Finally, H_2O_2 have an effect on PU degradation. Repeat treats the PUs catheter with Sterrad 100S® cause catheter surface oxidation.[15]

All the previous techniques have demonstrated efficiency to destroy microorganisms. However, multiple sterilization with those techniques may adversely affect the characteristics or quality of electophysicology catheters. That will lead to unsafe and ineffective use of catheters. At the best of our knowledge, there were no report relates to the toxicity of various sterilization methods on cardiac catheters. Therefore, we designed an *in vitro* model to evaluate toxicity of electrophysiology catheters after their repeated sterilization by steam autoclave, ethylene oxide, and Sterrad 100S®. Cytotoxicity of the potential leachables in the culture medium was evaluated after exposure of catheter extracts to macrophage in suspension.

6.3 Materials and Methods

6.3.1 Cell Culture

The J774 mouse macrophage cell line (ACTT, Rockville, MD, USA) was used as *in vitro* model. The macrophages were cultured in RPMI 1640 tissue culture medium (Biomedia Canada, Drummondville, Quebec, Canada) supplemented with 5% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37°C in a humidified atmosphere of 5 % CO_2 in air. Culture tubes without catheters and with unsterilized catheters served as negative control.

6.3.2 Polyurethane-Based Catheter

The catheters used in this study were new single-used electrophysiology polyurethane (PU)-based catheters manufactured by Cordis Corporation (a division of Johnson & Johnson Medical Products, Florida, USA). Samples were wrapped in plastic sterilization pouches and processed one or ten cycles by steam autoclave, ethylene oxide, and Sterrad-100S® system under hospital conditions. They were then subjected to 72 h extraction in RPMI 1640 tissue culture media at 37°C according to the Association for the Advancement of Medical Instrumentation (ISO 10993-5:1993). The ratio between the

surface area of the catheter and the extraction volume was 1.0 cm^2/mL. The extraction solution was used immediately after preparation.

6.3.3 Sterilization Procedure

Three sterilization methods were used in this study. All sterilizations were performed in hospital condition.

Steam autoclave Flash technique was used in this study. The samples were autoclaved for 4 min at 270°C in saturated humid atmosphere using a standard hospital autoclave (The Sir Mortiner B. Davis - Jewish General Hospital, Montreal). The catheters were then dry 20 min. For the 10 cycles of sterilization samples, catheters were treated 10 times with the same procedure.

Ethylene oxide EtO sterilization was performed at The Sir Mortimer B. Davis - Jewish General Hospital of Montreal in a Steri-Vac 3M system (London. ON, Canada). The catheters were exposed to with 60% humidity for 15 h at 55°C, then catheters pass aeration for 12 h. For the 10 cycles of sterilization samples, the same procedure was repeated 10 times. After sterilization, the samples were degassed for two weeks.

Hydrogen peroxide plasma The sterilization procedure (Sterrad-100S®) is divided into two phases. At the diffusion phase, catheters were exposed to hydrogen peroxide at 50°C

for 50 min. Then, catheters underwent a plasma phase lasting 15 min. After plasma cycle, samples were removed from the sterilization chamber to allow for possible short-term of aeration. For the 10 cycles of sterilization samples, catheters were treated 10 times with the same procedure (Hôpital Charle Lemoyne, Greenfield Park, Que, Canada).

6.3.4 Cytotoxicity Test

Extraction method was used for the cytotoxicity test according to the Association for the Advancement of Medical Instrumentation (ISO 10993-5:1993). Catheters were sterilized for one and ten cycles whereas unsterilized catheters served as control. The ratio of catheter with cell culture medium was 1.0cm^2/mL. Macrophage (5×10^5/mL) were incubated with the catheter extracts at 37°C for 24, 48 and 72 h. Viability of the macrophages were defined by the number of living cells divided by the total of living plus the dead cells using Trypan Blue exclusion. At the end of incubation, the culture media was collected and the concentration of TNF-α was measured by ELISA (BioSource, Nivelles, Belgium) as an indicator of macrophage activity. This assay kit is mouse specific with detection limits ranging from 5 to 1250 pg/mL.

6.3.5 Statistical Analysis

Results were the mean of four experiments realized in duplicate. Statistical significance was evaluated by the analysis of variance (ANOVA). A p value of <0.05 was considered significant.

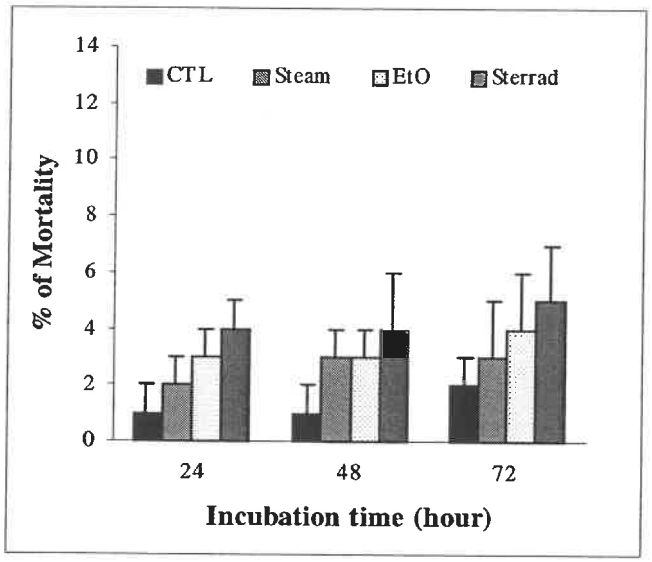
6.4 Results

6.4.1 Cytotoxicity

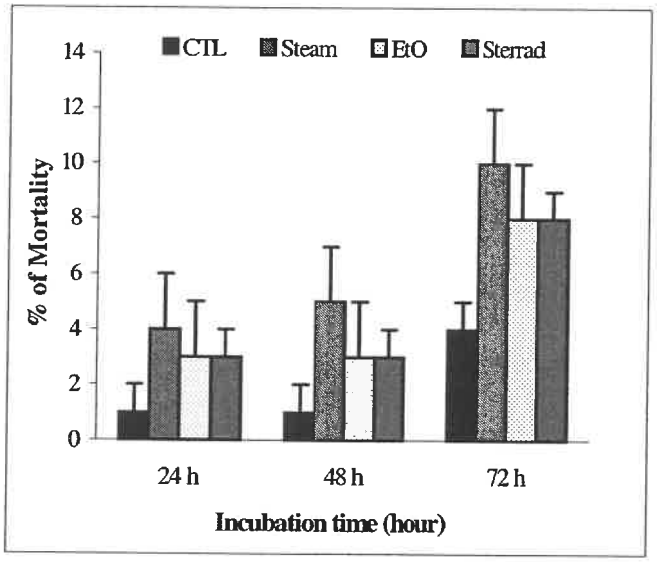
In a fist step, we tested the catheters that have undergone one cycle of sterilization. J774 macrophages were examined after a 24 h incubation with the 72 h extracts. The mortality of cells was less than 5%. The steam autoclave samples showed the lowest mortality (3%) whereas mortality attained 4 and 5% with EtO and Sterrad 100S® sterilized catheter extract, respectively (Figure 6.1). There were no significant difference between control and the three sterilization methods. After ten sterilization cycles, the steam autoclave samples shown the highest mortality ($10 \pm 1\%$) while extracts of catheters sterilized by EtO or Sterrad 100S® reached $8 \pm 2\%$. There was a significant difference in cell viability between extracts of control and sterilized samples ($p<0.05$), but no significant difference in cell viability among the three sterilization methods. In summary, the method of sterilization (steam, EtO, or Sterrad 100S®) had no effect on the viability of J774

macrophages whereas the number of sterilization (up to ten cycles) by EtO and Sterrad 100S® had a moderate effect on this viability.

However, we observed that the number of cells decreased in the presence of the catheter extracts. After incubation with unsterilized PU-based catheter extracts for 24 h, the number of cells reached 700 000 ± 76 000/mL while the number of cells was 560 000 ± 75 000/mL, 620 000 ± 57 000/mL, and 530 000 ± 77 000/mL with steam, EtO, and Sterrad 100S®, respectively. After 72 h incubation, the number of cells in control extracts increased to 990 000 ± 5 000/mL while this number reached 830 000 ± 3 000/ml, 800 000 ± 37 000/mL, and 740 000 ± 4 000/mL with steam, EtO, and Sterrad 100S® extracts, respectively (Figure 6.2). This represents a 14% to 38% significant decrease ($p<0.05$) in the number of cells. Moreover, the number of cells was significantly different between the three sterilization methods. However, there was no significant difference in cell number between one cycle and 10 cycles sterilized samples (Figure 6.3).



(a)



(b)

Figure 6.1. Effect of PU-based catheter extracts on macrophage mortality. J774 macrophages were incubated for 24-72h with catheter extracts. Extracts of non-sterilized catheter served as control. Results are the mean ± standard of 5 experiments. (a). 1 cycle of sterilization. (b). 10 cycles of sterilization.

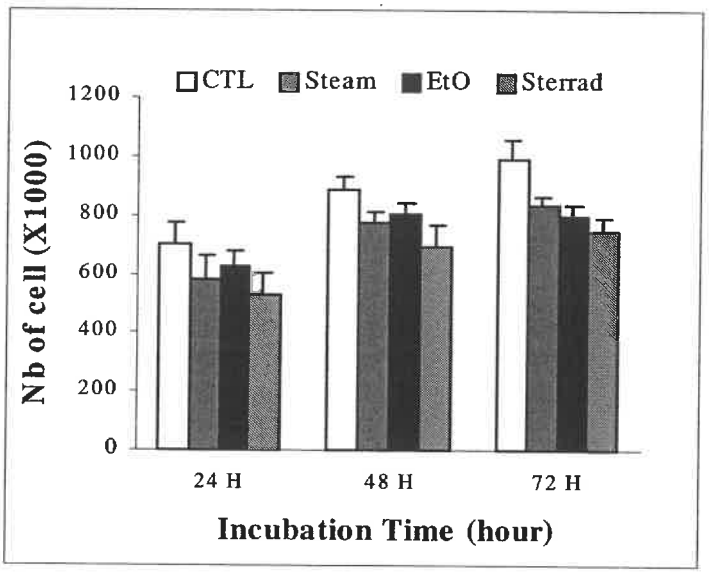


Figure 6.2. Effect of sterilization on the number of macrophages. J774 macrophages were incubated for 24-72 h with catheter extracts. Extracts of unsterilized catheters served as control. Results are the mean ± standard error of 5 experiments.

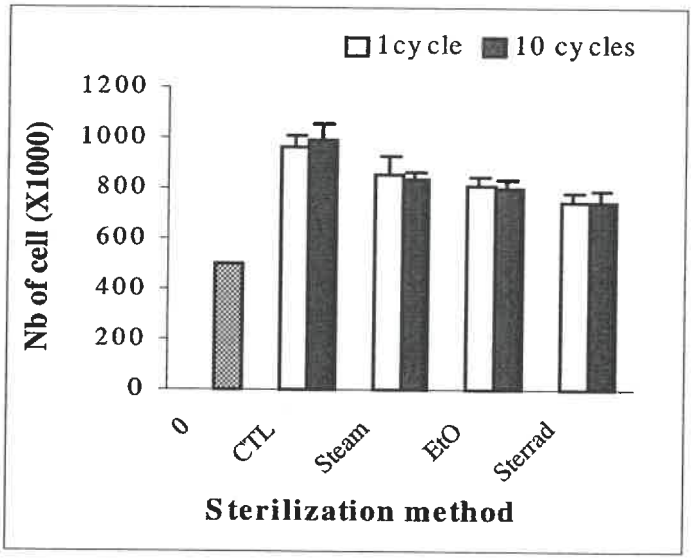
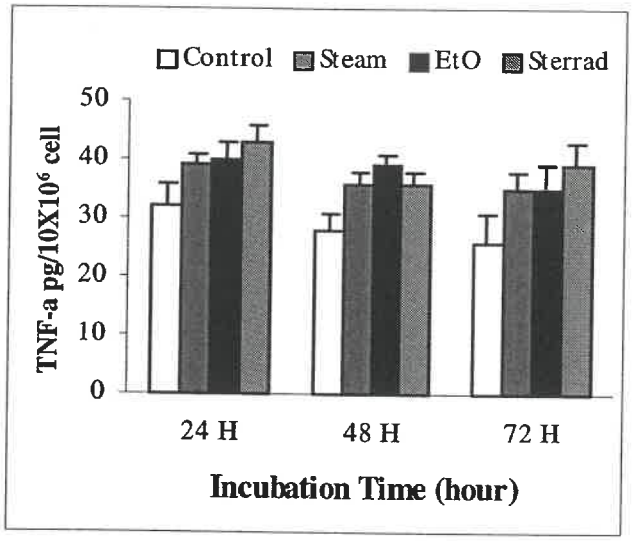


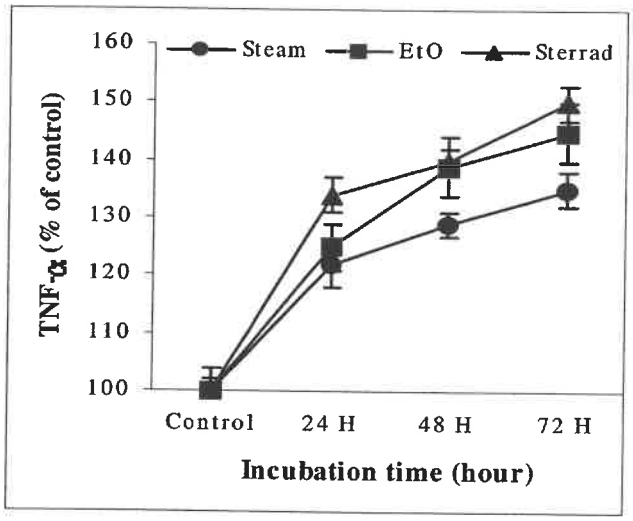
Figure 6.3. Effect of the number of sterilizations on macrophage growth. J774 macrophages were incubated for 24 h with catheter extracts. Extracts of unsterilized catheters served as control. Results are the mean ± standard error of 5 experiments.

6.4.2 TNF-α Release

In this study, TNF-α release was measured as an index of the activation of macrophages. Our results suggest that the extracts of sterilized PU-based catheters had no significant effect on the production of TNF-α by J774 macrophage (Figure 6.4). Indeed, TNF-α concentrations reached 122 ± 22 %, 125 ± 8 %, and 134 ± 5 % of control for steam, EtO, and Sterrad 100®, respectively. Once again, no significant difference was observed between sterilization methods and /or the number of sterilization cycles.



(a)



(b)

Figure 6.4. Effect of catheter extracts on TNF-α release. J774 macrophages were incubated for 24-72 h with catheter extracts. Extracts of unsterilized catheters served as control. The concentration of TNF-α was measured by ELISA. Results are the mean ± standard error of 4 experiments. (a) TNF-α concentration reported by pg/10x 10^6 cells. (b) TNF-α concentration reported by percentage of control.

6.5 Discussion

Our results suggest that sterilization of catheters have a weak cytotoxic effect on macrophages. In our experiments, the sterilized catheters were added directly into RPMI culture media and the extracts were used as conditioned media with J774 macrophages. Cells and catheters were not tangent to each other. So, the observed cytotoxicity was induced by agents leached out from the catheter and was not the results of cell-catheter interaction due to the physio-chemical property of the material surface.

More specifically, our study showed that the cytotoxicity was low with PU catheters sterilized by heat (steam). However, the repeated sterilization by heat increased the deleterious effect. Moreover, we observed a morphological change after 10 sterilization cycles by steam autoclave sterilization, the surface of catheter becoming rougher. PU surface properties were then modified during sterilization by steam at high temperature. It has been reported that PUs made from MDI are susceptible to degradation if processed in the presence of heat and if in an aqueous solution.[26] The release of low molecular weight compounds from PUs during autoclave sterilization has been also found as hydrolytic products of urine of patients with 7 month old PUs covered Meme breast implants.[27] The changes in surface morphology we observed in this study, might reflect PUs structure change. That will influence catheters efficiency. Therefore, we suggest that the best way to re-sterilization PU catheters is to avoid repeated use of high temperature.

The two low temperature sterilization methods we used in this study (EtO and Sterrad 100S®) seem to be more convenient for PU catheter re-sterilization in regard to the absence of morphologic change. However, according to the Food and Drug Administration standard regulation, the maximum permissible level of EtO for implantable products is 25 parts per million (ppm). The American Association for the Advancement of Medical Instrumentation lists hydrogen peroxide residuals as nontoxic and negligible.[29] It was reported that residual EtO level as high as 41 ppm after two days aeration and less than 25 ppm after 14 days aeration period.[28] Moreover, the amounts of residual H_2O_2 in the materials decreased with increasing aeration time. The greatest part H_2O_2 in PU materials can be removed by a 12 h aeration period of time.[25] In our study, catheters were set two weeks after sterilization by EtO or H_2O_2. Result showed no significant difference in cell viability between control and one cycle sterilized catheters. After ten sterilization cycles, we observed a significant difference ($p<0.05$) between these sterilization methods and the control condition. We think that change may not only be caused by chemical residues, but also by PU degradation products.

Indeed, it has been reported that Sterrad 100S® induces surface modifications on PU-based catheter.[15] This alteration can be explained by the oxidative effect of the sterilizing agent (H_2O_2) with an increase of crystallinity as a result of chain scission.[30] A preliminary study in our laboratory has demonstrated that Sterrad sterilization cause surface oxidation of all types of polymers.[31] Oxidation might induce PU compounds

release. These degradation products may affect on cell attachment and spreading. Multiple cycle sterilization by EtO and steam autoclave caused significant decreasing of cell number *in vitro* has been reported.[32] Our results demonstrated that Sterrad 100® also has inhibited effect on cell's growing. The rate of cell growth decrease in the presence of sterilized catheter extracts. There was no any correlation with sterilization method.

Cytokine secretion in the culture medium may be used as indicator of macrophage activation. In the present study, TNF-α release was higher with all sterilized PU-based catheter samples. This increase was however not significant. None of the sterilization methods used appeared to activate macrophage cytokine production related to inflammation. Therefore, we consider the changes in viability related to re-sterilization are not associated with an increased inflammatory response.

Reprocessing the medical devices includes two steps: cleaning and sterilization of the device. In our studies, we tested the potential toxic effect related to repeated sterilization only. However, others studies reported the safety of reusing catheters after careful cleaning and sterilization with EtO and hydrogen peroxide plasma.[33-36]

6.6 Conclusion

The result of this study shown that it is possible to measure the cytotoxicity of sterilization with mouse macrophage cell line. Our results demonstrate that multiple sterilized PUs-based catheters have no significant cytotoxic effect on J774 macrophages. The cytotoxicity was however low without any correlation with extraction time or number incubation. Finally, extracts of PU-based catheters had no effect on TNF-α release by J774 macrophage. These findings may suggest that re-sterilized electrophysiology catheters are biological acceptable. If the functional characteristics of the catheter do not change, PU-based catheters can be potentially considered for repossessing in hospitals.

Acknowledgement

This study was supported by National Science and Engineering Research Council (NSERC). The authors would like to thank Hôpital Charles Lemoyne and Jewish General Hospitals of Montréal for catheter sterilization.

No benefit of any kind will be received either directly or indirectly by the author(s).

6.7 References

1. Conseil d'évaluation des technologies de la santé du Québec. The reuse of single-use cardiac catheters: safety, economical, ethical and legal issues. Can J Cardiol. 1994;10: 413-421.

2. Dunnigan A, Roberts C, McNamara M, Benditt D. Success of re-use of cardiac electrode catheters. Am J Cardiol 1987;60:807-810.

3. Browne K F, Maldonado R, Telatnik M, Vlietstra R E, Brenner A S. Initial experience with reuse of coronary angioplasty catheters in the United States. J Am Coll Cardiol 1997; 30:1735-1740.

4. Report from the Canadian Coordinating Office for Health Technology. Assessment (CCOHTA). Reuse of single-use cardiac catheters. Int J Technol Assess Health Care 1991;7: 637-638.

5. Avitall B, Khan M, Krum D, Jazayeri M, Hare J. Repeated use of ablation catheters: a prospective study. J Am Coll Cardiol. 1993; 22:1367-1372.

6. Lelah M D, Cooper L S. Polyurethane in medicine. CRC Press. Boca Raton, FL. 1986. P 22-33.

7. Szycher M, Siciliano AA., Reed AM. Polyurethanes in medical devices. Med Res Mater 1991;1(2):18-25.

8. Lamba NMK, Woodhouse KA, Cooper SL. Polyurethanes in biomatedical applications. CRC Press. Boca Raton, FL .1998. p 205-208.

9. Shntani H. Formation and elution of toxic compounds from sterilized medical products: methylenedianiline formation in polyurethane. J Bioma Appli 1995;10: 23-36.

10. Nair PD. Currently practiced sterilization methods-Some inadvertent consequences. J Biomater Appl 1995;10:121-135.

11. Pinchuk L. A review of the biostability and carcinogenicity of polyurethanes in medicine and the new generation of 'biostable' polyurethanes. J Biomateri Sci, Polym Ed 1994:6:225-267.

12. Okogel T, Soldani G, Goddard M, Galletti PM. Penetrating micropores increase patency and achieve extensive endothelialization in small diameter polymer skin coated vascular grafts. Trans Am Soc Artif Intern Organs 1996;42:M398-401.

13. Gogolewski S. Implantable segmented polyurethanes: controversies and uncertainties. Life Support Syst 1987;5: 41-46.

14. Jayabalan M and Lizymol PP. Effect of autoclaving sterilization in the stability of polyurethane potting compounds based on caprolactone polyol. J Polymer Material 1997; 14:49-55.

15. Lerouge S, Guignot C, Tabrizian M, Ferrier D, Yagoubi N, Yahia L'H. Plasma-based sterilization: Effct on surface and bulk properties and hydrolytic stability of reprocessed polyurethane electrophysiology catheters. J Biomed Mater Res 2000;52:774-782.

16. Pizzoferrato A, Arciola CR, Cenni E, Ciapetti G and Sasi S. In vitro biocompatibility of a polyurethane catheter after deposition of fluorinated film. Biomaterials 1995;16: 361-367.

17. Goullet D. La stérilisation des biomatériaux: quelle méthode choisir. *Agressologie.* 1992; 33: 121-123.

18. Black SS.: Disinfection sterilization and preservation. Lea & Febiger 1991:495-512.

19. Zdrahala RJ. Small caliber vascular grafts. Part II. Polyurethane revisited. J Biomater Appl 1996;11:37-61.

20. Nair PD. Currently practised sterilization methods-some inadvertent consequences. J Bioma Appli 1995; 10:121-135.

21. National Toxicology Program. Toxicology and carcinogenesis studies of ethylene oxide (CAS No. 75-21-8) in B6351mice (inhalation studies). Technical report series no 326. Research triangle Park, NC: U.S. Department of Health and Human Services. Public Health Service, National Institutes of Health. (NIH publication no.88-2582).

22. Aton EA, Murray P, Fraser V, Conaway L, Cain M. Safety of reusing cardiac electrophysiology catheters. Am J Cardiol 1994; 74:1173-1175.

23. Bathina MN, Mickelsen S, Brooks C, Jaramillo J, Hepton T, Kusumoto FM. Safety and efficacy of hydrogen peroxide plasma sterilization for repeated use of electrophysiology catheters. J Am Coll Cardiol 1998;32:1384-1388.

24. Kyi MS, Holton J, Ridgway GL. Assessment of the efficacy of a low temperature hydrogen peroxide gas plasma sterilization system. J Hosp Infect 1995; 31:275-284.

25. Ikarashi Y, Tsuchiya T, Nakamura A. Cytotoxicity of medical materials sterilized with vapour-phase hydrogen peroxide. Biomaterials 1995;16:177-183.

26. Mazzu AL, Smith CP. Determination of extractable methylene dianiline in thermoplastic polyurethanes by HPLC. J Biomed Mater Res 1984;18:961-968.

27. Chan SC, Birdsell DC, Gradeen CY. Detection of toluenediamines in the urine of a patient with polyurethane-covered breast implants. Clin Chem 1991;37:756-758.

28. Ferrell M, Wolf CE 2nd, Ellenbogen KA, Wood MA, Clemo HF, Gilligan DM. Ethylene oxide on electrophysiology catheters following resterilization: implications for catheter reuse. Am J Cardiol 1997; 80:1558-1561.

29. AAMI TIR No.12. Designing, Testing, and Labeling Reusable Medical Devices for Reprocessing in Health Care Facilities. 1994;VII: 146.

30. Goldman M, Gronsky R, Long GG, Pruitt L. The effects of hydrogen peroxide and sterilization on the structure of ultrahigh molecular weight polyethylene. Polymer Degradation and Stability 1997;62:97-104.

31. Tabrizian M, Lerouge S, Wertheimer MR., Marchand R, L'Hocine Y. Surface modification of polymer-based devices by cold sterilization techniques. Proceedings of the First International Symposium on Advanced Biomaterials, 1997; Montréal, Québec, Canada.

32. Vezeau PJ, Koorbusch GF, Draughn RA, Keller JC. Effect of multiple sterilization on surface characteristics and in vitro biologic responses to titanium. J Oral Maxillofac Surg. 1996;738-746.

33. Fagih B, Eisenberg MJ. Reuse of angioplasty catheters and risk of Creutzfeldt-Jakob disease. J Am Heart 1999;137:1173-1178.

34. Vink P, Pleijsier K. Aeration of ethylene oxide-sterilized polymers. Biomaterials 1986; 7:225-230.

35. Guidoin R, Snyder R, King M, Martin L, Botzko K, Awad J, Marois M, Gosselin C. A compound arterial prosthesis: the importance of the sterilization procedure on the healing and stability of albuminated polyester grafts. Biomaterials 1985;6:122-128.

36. Penna TC., Ferraz CA: Cleaning of blood-contaminated reprocessed angiographic catheters and spinal needles. Infect Control Hosp Epidemiol 2000; 21:499-504.

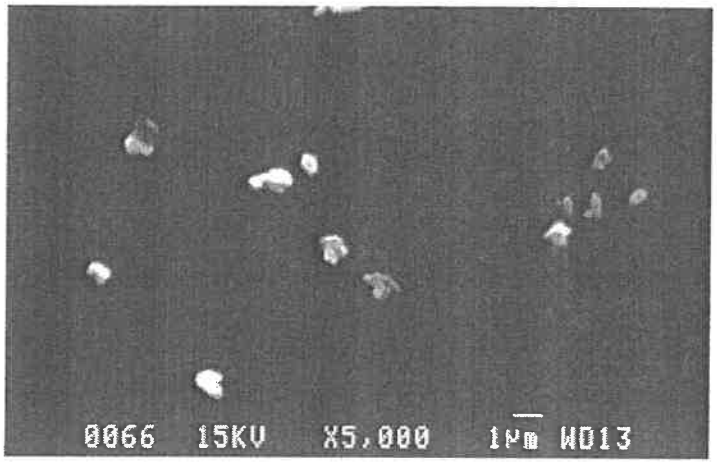
CHAPTER 7. GENERAL DISCUSSION

The general aim of the present study was to investigate the potential cytotoxic effects of PUs used in clinical applications. We used PUs in the forms of catheters and particles. Our results demonstrated that macrophage response to resterilized catheters is low while their response to particles is relatively stronger.

7.1 Cytotoxicity of Polyurethane Particles

The thermoplastic found in Pellethane 2363® represents the most typical PU structure used in medical devices for more than thirty years (Szycher, 1991). This PU is made from a base of MDI-BD hard segments combined with PTMO soft segments. This combination provides excellent physical properties as well as high blood and tissue compatibility. As mentioned in Chapter 2, the problem associated with PU devices of the Pellethane® 2363 serie is surface cracking (Stokes *et al.*, 1987; Chawla *et al.*, 1988). The nature of the PU used and the activation of macrophages have been considered the most important parameters playing a role in this phenomenon. We employed an *in vitro* model to study the interaction of commercially available PUs (Pellethane® and Tecothane®) with macrophages. Since it is difficult to obtain biomaterials from their *in vivo* environment, we produced particles from PU beads by a modified solvent vapor method. Under scanning electronic microscope (SEM), the morphological characteristics of these

particles were found to be similar to those previously observed *in vivo* (Szycher, 1991) (Figure 7.1).



(a)

(b)

Figure 7.1. Comparison of our own PU particle (a) to polyurethane particles that were observed *in vivo* (b). (Szycher, 1991. *In vivo* testing of a biostable polyurethane. *J. Biomater. Appl.* 6, 110-130).

Even though it is commonly accepted that the release of components from biomaterials plays an important role in regulating inflammatory responses, the overall process is not clearly defined (Zhao *et al.*, 1993). Our results indicate that J774 macrophages are sensitive to the presence of PU particles. Two parameters influenced their viability: the concentration of particles and the type of material. The influence of particle concentration was obvious in all tested samples. Indeed, cell mortality increased with the increase of particle concentration. However, the three different types of particles exhibited different cytotoxic effects. PL has a relative weak effect on the cell mortality. After 24h and 48h of incubation, the mortality of cells incubated with TC85 particles was higher than that produced with PL samples, but lower than the mortality induced by TC65 particles.

The concentration and type of particles also decreased the rate of cell growth. Since any components of PUs can potentially be toxic for cells, it is not surprising that cytotoxic effects are associated with high particle concentrations. This phenomenon has already been observed with other biomaterials, such as polyethylene and ceramic (Catelas *et al.*, 1998). Our results suggest that it would be beneficial to minimize degradation in order to ameliorate long-term PU device biocompatibility. Moreover, particular attention should be taken in the choice of PU because composition affects cell mortality and growth.

Identification of the behaviors of macrophages in contact with PU particles is important since they trigger chronic inflammations through the release of soluble mediators such as

cytokines, free radicals, and degradative enzymes. The measurement and analysis of cytokine release is then become important parameters that reflect cell response and prolongation of the inflammation (Panichi *et al.*, 2000). Our results showed that the three types of PU particles stimulate macrophage TNF-α release, especially during the first 24 h of incubation. The concentration of TNF-α release was, again, dependent on the concentration and the type of PU particles. Overall, TNF-α release was higher in the presence of TC65 particles than in the presence of TC85 and PL particles. These results support those of cytotoxicity and strongly suggest that TNF-α release can be used as a marker of macrophage response to PUs *in vitro*.

Our results also suggest that PU leachable products cause macrophage activation, which is associated with cytokine production. This production may increase the stimulation of macrophages (and/or neutrophils) accumulation at the surface of devices or surrounding tissues, resulting in prolonged chronic inflammation as well as biodegradation of PU devices. Over time, the changes in the material may elicit a change in cell response because of a cross-talk reaction.

Our results clearly demonstrate that the cytotoxicity (cell mortality and TNF-α release) of Tecothane® was higher than that of Pellethane®, particularly for TC65. Since the sizes and morphologies of these particles were greatly similar, the only difference between these particles is the ratio of their hard/soft segments. Based on ^{1}H-NMR spectra, the ratio of hard/soft segments were 35%, 37%, and 58% for PL, TC85 and TC65,

respectively. Our results demonstrate no significant difference in cytotoxicity between PL and TC85, which contain comparable hard/soft segment composition. However, TC65 particles, containing the highest percentage of hard segments, also showed the highest cytotoxicity. Brunstedt *et al.* (1990) reported that cellular activities differed with the type of Pellethanes. The authors also observed an increase of macrophage adherence, cytoplasmic spreading, and macrophage membrane fusion with increasing Pellethane hardness. The influence of hard segments has been highlighted in other studies. Previous authors have reported the effect of polymer hard segments on cell reaction (Casas *et al.*, 1999) and PU degradation (Tyler *et al.*, 1994). The hard fragments of aromatic structures and their bioactive products, such as TDA and MDA, were detected on the PU implant surface and associated with carcinogenicity (Daka *et al.*, 1993; Chawla *et al.*, 1988; Tsuchiya *et al.*, 1996).

7.2 Cytotoxicity of Resterilized Catheters

The reuse of electrophysiology catheters after reprocessing has been under discussion for a long time. For example, several studies investigated the effects of re-sterilization on the efficiency and physical properties of catheters (Bathina *et al.*, 1998; Bentolila, 1990; Hoffmann *et al.*, 2000). However, few publications have addressed the potential toxicity related to the re-sterilization of polymers (Aton *et al.*, 1994; Conseil d'Évaluation des

Téchnologies de la santé du Québec, 1994). Moreover, there are no established norms or protocols for polymer re-sterilization procedures and for reuse.

We chose the EtO and Sterrad 100S® sterilization methods since they are the most commonly used techniques in hospitals. High temperature sterilization, i.e. by steam, was the earliest method applied to sterilize biomaterials and is still used today. Even if this method is not recommended for the sterilization of PUs, it served as a reference for comparison purposes in this study.

We observed that the physical appearance of catheters changed drastically after ten cycles of treatment by steam autoclave (Figure 7.2). The surface of the catheter became rough and lost its smooth appearance. There were no visible changes on the surface of the catheters treated with the low temperature sterilization methods (EtO and Sterrad). The observed effect of steam on the physical appearance of PU is not surprising since they are known to be sensitive to heat and moist environments (Darby *et al.*, 1978). On the other hand, our results suggest that re-sterilization of catheters, for up to 10 cycles, does not cause significant morphological changes of the catheters after treatment with the low temperature techniques.

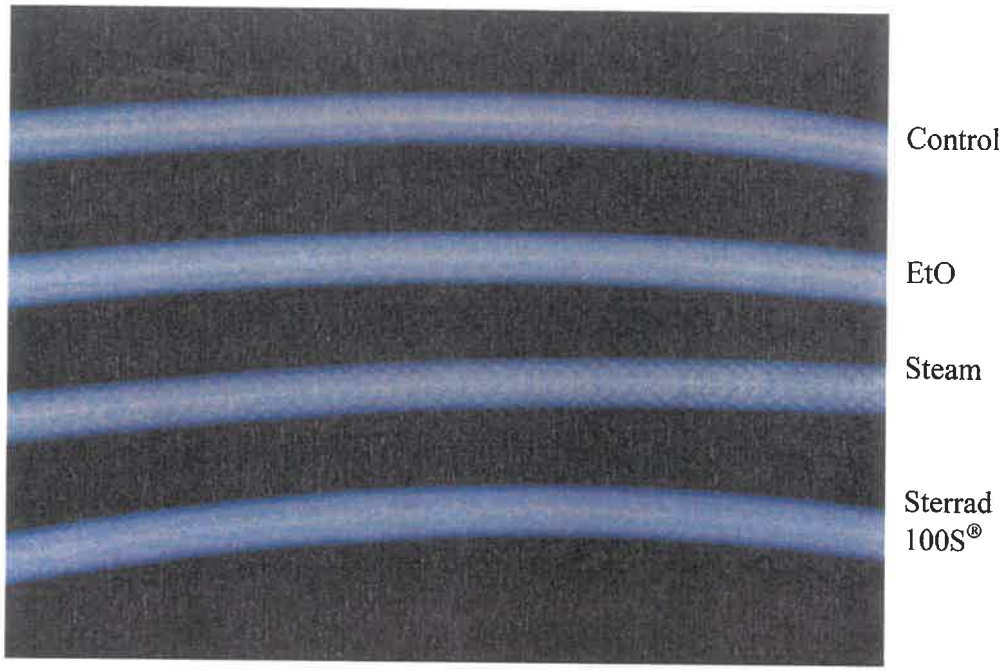


Figure 7.2. Catheters after ten cycles of sterilization.

We measured the macrophage response to extracts from catheters that were sterilized by different methods. Our results showed that the overall cytotoxicity due to re-sterilization is low. However, two parameters appeared to influence cellular response, even if not significantly: the number of sterilization cycles and the method of sterilization. Indeed, our results demonstrated that after one cycle of re-sterilization, catheters treated by steam autoclave had the lowest cytotoxic effect while the catheters sterilized by steam autoclave for ten cycles had the highest cytotoxicity. Previous studies have demonstrated that catheter sterilization in heated and humid environments cause PU chain cleavage as well

as release of toxic substances (Habermann *et al.*, 1985; Mazzu *et al.*, 1984; Szycher, 1985) and particles to their surface (Karov *et al.*, 2000). Furthermore, cracks and particle wrinkles were also observed by scanning electron microscopy on angioplasty catheter balloons (Karov *et al.*, 2000). These results strongly suggest that steam autoclave is not suitable for the multiple sterilization of PU catheters.

On the other hand, our results demonstrated an increased macrophage mortality with the number of sterilization with EtO and Sterrad 100S®, without significant differences between the two methods. Sterilization by EtO produces breakdown products of ethylene glycol, ethylene chlorohydein, and dioxane (Buben, 1999), all potentially toxic for humans (Health Industry Manufactures Association, 1991). Therefore, the level of residual EtO should be kept as low as possible. FDA recommends a maximum level of 25 parts per million (ppm). A recent study showed that resterilized electrophysiology catheters had residual ethylene oxide level (41 ppm) exceeding this FDA limit (Ferrell *et al.*, 1997). This level can be reduced below 25 ppm when a 14-day aeration time is included in the process. In our study, 12 h of aeration time was allowed between each cycle of sterilization with another 14 days of aeration after the last cycle. The slightly increase in mortality observed after 10 cycles of sterilization suggests that a certain amount of chemicals may accumulate on the surface of catheters (4% to 8% of cell mortality incubated with EtO sterilized catheters).

A similar cytotoxicity was observed after 10 cycles of sterilization by Sterrad 100S®. This vapour-phase H_2O_2 sterilization process was developed recently. Few studies evaluated its potential toxicity. Meijis *et al.* (1993) reported a H_2O_2 oxidative effect on medical grade PUs. Indeed, gel permeation chromatography and infrared spectroscopy studies showed that the treatment of PUs with hydrogen peroxide led to a significant decrease in their mechanical properties accompanied by a reduction in molecular weight as well as a decrease in polyether soft segments at the surface. A study performed in our group demonstrated that Sterrad sterilization caused surface oxidation of all types of polymers (Lerouge *et al.*, 2000). This might explain the high decrease in cell number observed in our study. No previous studies have reported this effect of H_2O_2.

In the present study, TNF-α release was used as an indicator of macrophage activation. The release of TNF-α was slightly higher with the sterilized PU-based catheter extracts compared to the control extracts where catheters were not sterilized. However, this increase was not significant. We therefore believe that the increased cell mortality associated with re-sterilization procedures is not related to macrophage activity.

In conclusion, our findings suggest that, if enough aeration time is allowed and the appropriate method is applied, resterilized electrophysiology catheters may be biologically acceptable for clinical use. However, we did not test the potential cytotoxic effect of catheter cleaning. Most reprocessings of used catheters are carried out by first cleaning them with deionized water and then with a H_2O_2 solution to remove any

proteinous matter. Since H_2O_2 is a strong oxidizing agent, the levels of toxic substances in catheters cleaned and resterilized should be higher than those attained after re-sterilization only.

Furthermore, cytotoxicity is not the only factor that should be considered when choosing a method for catheter sterilization. The preservation of the physical and functional properties of catheters is another important factor in the identification of the most adequate method of sterilization. Multiple sterilizations may negatively affect the mechanical integrity, flexibility as well as deflection of electrophysiology catheters. Any significant changes of these properties would adversely affect the safety and efficiency of the catheter. Therefore, maintenance of the mechanical properties of PUs after re-sterilization must be considered before clinical applications.

CONCLUSION AND PERSPECTIVES

The present master degree work evaluated the macrophage response to PU materials by evaluating the cytotoxic effects of re-sterilized PU catheters and PU particles.

Our results clearly demonstrated a relation between PU chemical structure and cytotoxic risk. This finding is important because although several studies mentioned the cytotoxicity of PU components, very few paid attention to hard/soft segment ratios on cell response. Based on our results, it is possible to decrease macrophage activation by either modifing the materials to decrease their component release as low as possible and by an adequate selection of hard segment and soft segments.

By re-sterilization process of PU-based electrophysiology catheters, we demonstrated a very low cytotoxic risk. This should be helpful for government or related agency in the formulation of a policy for re-sterilization and re-use of cardiac catheters. However, further work is necessary to investigate the potential cytotoxic effect of the whole re-processing, including cleaning and sterilization.

Further studies are also necessary to investigate the phagocytosis of PU particles by macrophages. This can be achieved for example by flow cytometry that represent a sensitive powerful tool. Since the proliferation of cells seems affected by PU particles,

more specific methods, such as estimated cell number by DNA content by enzyme-linked immunosorbent assay, or by the antibodies, are necessary to confirm PU inhibitory effect on cell growing. Cell mortality can be divided into two general processes, necrosis and apoptosis. Necrosis is a passive form of cell death generally induced by high concentration of toxic agents. Apoptosis is an active form of cell death and requires the participation of cellular processes including new protein synthesis. The recent explosion of interest in apoptosis lies in the fact that it is a genetically controlled phenomenon and in the fact that the elimination of apoptotic cells by phagocytes can prevent an inflammatory response. In our study, we explored the effect of PU particles on apoptosis. Our preliminary data by Western blot showed that PU particles did not induce poly (ADP- ribose) polymerase cleavage, a marker of apoptosis. However, several cellular mechanisms are responsible for the induction of apoptosis and, therefore, only one technique is insufficient to prove the presence or the absence of apoptosis. The induction (or not) of apoptosis by PU particles should be achieved by the visualization of DNA laddering that measure the fragmentation of DNA into oligonucleosome-sized fragments, a gold standard in the study of apoptosis. The measurement by flow cytometry of Annexin V binding on the extra cellular membrane of cells is a rapid and sensitive method to detect early apoptosis that should also be used to prove the induction (or not) of apoptosis by PU particles. Finally, it will be of great importance to perform the same kind of experiments on all new polyurethane products.

While PU materials continue to be attractive for biomedical application, new research efforts should be based on the understanding of the mechanisms of failure of PU devices. The stability of implanted biomaterials can only be predicated if the interactions between inflammatory cells and the biomaterials are clearly understood. Once these mechanisms will be known, it should be possible to produce biomaterials that trigger minimal inflammation or sufficiently durable to withstand the responses of the host.

REFERENCES

AAMI TIR No.12. (1994). Designing, Testing, and Labeling Reusable Medical Devices for Reprocessing in Health Care Facilities. VII: 146.

ADEREM A. and UNDERHILL D.M. (1999). Mechanisms of phagocytosis in macrophages. Annu.Rev.Immunol., 17, 593-623.

ALGAN S. M., PURDON M, HOROWITZ S.M. (1996). Role of tumor necrosis factor alpha in particulate-induced bone resorption. J. Orthop. Res., 14, 30-35.

ALI S.A.M., DOOHERTY P.J., and WILLIAMS D.F. (1994). Molecular biointeractions of biomedical polymers with extracellular exudate and inflammatory cells and their effects on the biocompatibility, in vivo. Biomaterials., 15, 779-785.

ANDERSON J.M. and MILLER K.M. (1984). Biomaterial biocompatibility and the macrophage. Biomaterials, 5, 5-10.

ANDERSON J.M. (1988). Inflammatory response to implants. ASAIO Trans., 34, 101-107.

ANDERSON J.M., Hiltner A., Wiggins M.J., Schubert M.A., Collier T.O., Kao W.J., and Mathur A.B. (1998). Recent Advaces in Biomedical Polyurethane Biostability and Biodegradation. Polymer International, 46, 163-171.

ASHLEY F.R. (1970). A new type of breast prosthesis. Preliminary report. Plastic Reconstr. Surg., 45, 421.

ATON E.A., MURRAY P., FRASER V., CONAWAY L., and CAIN M. (1994). Safety of reusing cardiac electrophysiology catheters. Am. J. Cardiol., 74, 1173-1175.

AVITALL B., KHAN M., KRUM D., JAZAYERI M., and HARE J. (1993). Repeated use of ablation catheters: a prospective study. J. Am. Coll. Cardiol., 22, 1367-1372.

BATHINA M.N., MICKELSEN S., BROOKS C., JARAMILLO J., HEPTON T., and KUSUMOTO F.M. (1998). Safety and efficacy of hydrogen peroxide plasma sterilization for repeated use of electrophysiology catheters. J. Am. Coll. Cardiol., 32, 1384-1388.

BATICH C., WILLIAMS J., and KING R. (1989). Toxic hydrolysis product from a biodegradable foam implant. J. Biomed. Mater. Res., 23, 311-319.

BAYER O. (1947). The diisocyanate polyaddiotion process (polyurethane). Description of a new principle for building up high-molecular compounds. Angew.Chem., A59, 275.

BEAMAN L. And BEANMAN B.L. (1984). The role of oxyzen and its derivatives in microbial pathogenesis and host defense. Ann. Rev. Microbiol., 38, 27-48.

BENOIT F.M. (1993). Degradation of polyurethane foams used in the Meme breast implant. J. Biomed. Mater. Res., 27, 1341-1348.

BENTOLILA P., JACOB R., and ROBERGE F. (1990). Effects of re-use on the physical characteristics of angiographic catheters. J. Med. Eng. Technol., 14, 254-259.

BENZ E.B., SHERBURNE B., HAYEK J.E., FALCHUK K.H., SLEDGE C.B., and SPECTOR M. (1996). Lymphadenopathy associated with total joint prostheses. A report of two cases and a review of the literature. J. Bone. Joint. Surg. Am., 78, 588-593.

BORETOS J.W. and PIERCE W.S. (1967). Segmented polyurethane: a new elastomer for biomedical applications. Science, 158, 1481-1482.

BOUVIER M., CHAWLA A.S., and HINBERG I. (1991). In vitro degradation of a poly(ether urethane) by trypsin. J. Biomed. Mater. Res., 25, 773-789.

BROWNE K. F., MALDONADO R., TELATNIK M., VLIETSTRA R.E., and BRENNER A.S. (1997). Initial experience with reuse of coronary angioplasty catheters in the United States. J. Am. Coll. Cardiol., 30, 1735-1740.

BRUCK S. D. (1991). Biostability of materials and implants. J. Long Term Eff. Med. Implants, 1, 89-106.

BRUNSTEDT M.R., ANDERSON J.M., SPILIZEWSKI K.L., MARCHANT R.E. and HILTNER A. (1990). In vivo leucocyte interactions on Pellethane surfaces. Biomaterials, 11,370-378.

BUBEN I., MELICHERCIKOVA V., NOVOTNA N., and SVITAKOVA R. (1999). Problems associated with sterilization using ethylene oxide. Residues in treated materials. Cent Eur J Public Health., 7, 197-202.

CANADIAN COORDINATING OFFICE FOR HEALTH TECHNOLOGY ASSESSMENT. (1991). Reuse of single-use cardiac catheterts. Ottawa: Canadian Coordinating Office for Health Technology Assessment.

CANADIAN STANDARD ASSOCIATION (a). (1985). Effective sterilization in hospitals by the ethylene oxide process (standard CAN3-Z314.2-M84). Rexdale: Canadian standards Association. 4.2.1.3.

CARSON RJ, EDWARDS A. and SZYCHER M (1996). Resistance to biodegradative stress cracking in microporous vascular access grafts. J. Biomater. Appl., 11, 121-134.

CASAS J., ZHAO Q, DONOVAN M., SCHROEDER P., STOKES K. and UNTEREKER D. (1999). In vitro modulation of macrophage phenotype and inhibition of polymer degradation by dexamethasone in a human macrophage/Fe/stress system. J. Biomed. Mater. Res., 46, 475-484.

CATELAS I., PETIT A., MARCHAND R., ZUKOR D.J., YAHIA L. and HUK O.L. (1998). Cytotoxicity and macrophage cytokine release induced by ceramic and polyethylene particles in vitro. J. Bone Joint Surg. Br., 81, 516-521.

CHAN S.C., BIRDSELL D.C. and GRADEEN C.Y. (1991). Detection of toluenediamines in the urine of a patient with polyurethane-covered breast implants. Clin Chem., 37, 756-758.

CHAWLA A.S., BLAIS P., HINBERG I., and JOHNSON D. (1988). Degradation of explanted polyurethane cardiac pacing leads and of polyurethane. Biomater Artif Cells Artif Organs., 16, 785-800.

CLARK R.A.F. and HENSON P.M. (1988). The molecular and cellular biology of wound repair. Plenum Press, New York, USA.

COLLIER T., TAN J., SHIVE MM HASAN S, HILTNER A., and ANDERSIN J. (1998). Biocompatibility of poly(etherurethane urea) containing dehydroepiandrosterone. J. Biomed. Mater. Res., 41, 192-201.

CONSEIL D'ÉVALUATION DES TECHNOLOGIES DE LA SANTÉ DU QUÉBEC. (1994). The reuse of single-use cardiac catheters: safety, economical, ethical and legal issues. Can. J. Cardiol., 10, 413-421.

DARBY T.D., JOHNSON H.J. and NORTHUP S.J. (1978). An evaluation of a polyurethane for use as a medical grade plastic. Toxicol .Appl. Pharmacol., 46, 449-453.

DAKA J.N. and CHAWLA A.S. (1993). Release of chemicals from polyurethane foam in the Meme breast implant. Biomater Artif Cells Immobilization Biotechnol., 21, 23-46.

DEVOR D.E., WAALKES M.P., GOERING P. and REHM S. (1993). Development of an animal model for testing human breast implantation materials. Toxicol. Pathol., 21, 261-273.

DUMITRIU S. (1994). Polymeric Biomaterials. Marcel Dekker, Inc., 233-244.

DUNNIGAN A., ROBERTS C., MCNAMARA M., and BENDITT D. (1987). Success of re-use of cardiac electrode catheters. Am. J. Cardiol., 60, 807-810.

EASTMAN A: Apoptosis (1993). A product of programmed and unprogrammed cell death. Toxicol Appl Pharmacol., 121, 160-164.

FAGIH B. and EISENBERG M.J. (1999). Reuse of angioplasty catheters and risk of Creutzfeldt-Jakob disease. J. Am. Heart., 137, 1173-1178.

FERRELL M., WOLF C.E. 2nd, ELLENBOGEN K.A., WOOD M.A., CLEMO H.F., and GILLIGAN D.M. (1997). Ethylene oxide on electrophysiology catheters following re-sterilization: implications for catheter reuse. Am. J. Cardiol., 80, 1558-1561.

GOLDMAN M., GRONSKY R., LONG G.G., and PRUITT L. (1997). The effects of hydrogen peroxide and sterilization on the structure of ultrahigh molecular weight polyethylene. Polymer Degradation and Stability, 62, 97-104.

GORBET M.B, YEO E.L., and SEFTON M. V. (1999). Flow cytometric study of in vitro neutrophil activation by biomaterials. Biomed Mater Res., 5, 289-297.

GOULLET D. (1992). La stérilisation des biomatériaux: quelle méthode choisir. Agressologie, 33, 121-123.

GRASEL T.G. and COOPER S.L. (1986). Surface properties and blood compatibility of polyurethaneureas. Biomaterials, 7, 315-322.

GUIDOIN R., SNYDER R., KING M., MARTIN L., BOTZKO K., AWAD J., MAROIS M. and GOSSELIN C. A. (1985). A compound arterial prosthesis: the importance of the sterilization procedure on the healing and stability of albuminated polyester grafts. Biomaterials, 6, 122-128.

GUIDOIN R., THERRIEN M., ROLLAND C., KING M., GRANDMAISON J.L., KALIAGUINE S., BLAIS P., PAKDEL H. and ROY C. (1992). The polyurethane foam covering the Meme breast prosthesis: a biomedical breakthrough or a biomaterial tar baby? Ann. Plast. Surg., 28, 342-353.

HABERMANN V. and WAITZOVA D. (1985). On the safety evaluation of extracts from synthetic polymers used in medicine. Arch. Toxicol. Suppl., 8, 458-460.

HARADA Y., WANG J.T., DOPPALAPUDI V.A., WILLIS A.A., JASTY M., HARRIS W.H., NAGASE M., and GOLDRING S.R. (1996). Differential effects of different forms of hydroxyapatite and hydroxyapatite/tricalcium phosphate particulates on human monocyte/macrophages in vitro. J. Biomed. Mater. Res., 31: 19-26.

HERMAN S.(1984). The Meme implant. Plast .Reconstr. Surg., 73, 411-414.

HELLER R.A. and KRONKE (1994). Tumor necrosis factor receptor-mediated signalin pathways. J. Cell Biol., 126, 5-9.

HINRICHS W.L., KUIT J., FEIL H., WILDEVUUR C.R., and FEIJEN J. (1992). In vivo fragmentation of microporous polyurethane- and copolyesterether elastomer-based vascular prostheses. Biomaterials, 13, 585-593.

HOFFMAN D, GONG G, PINCHUK L and SISTO D. (1993). Safety and intracardiac function of a silicone-polyurethane elastomer designed for vascular use. Clin. Mater., 13, 95-100.

HOFFMANN R., HAAGER P., MINTZ G., and KLUES H. (2000). Mechanical properties and imaging characteristics of remanufactured intravascular ultrasound catheters. Int J Card Imaging., 16, 23-27.

HOLEVINSKY K. O., NELSON D. J. (1998). Membrane capacitance changes associated with particle uptake during phagocytosis in macrophages. Biophys J., 75, 2577-2586.

HUNT J. A, FLANAGAN B. F, MCLAUGHLIN P. J, STRICKLAND I. and WILLIAMS D. F. (1996). Effect of biomaterial surface charge on the inflammatory

response: Evaluation of cellular infiltration and TNF-α production. J. Biomed. Mater. Res., 31, 139-144.

IKARASHI Y., TSUCHIYA T. and NAKAMURA A. (1995). Cytotoxicity of medical materials sterilized with vapor-phase hydrogen peroxide. Biomaterials, 16, 177-183.

INSTITUTE OF HEALTH POLICY ANALYSIS. (1986). Results of an informal survey on reuse. Institute fr health ploly analysis . Survey Results-Appendix B, Panel Report. Reuse of Disposable Mediacal Devices: Legal and Public Polycy Issue. B1-5.

JANICKE R. U, SPRENGART M. L, WATI M. R and PORTER A. G. (1998). Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J Biol Chem., 273, 9357-9360.

JAYABALAN M. (1993). Biological interactions: Causes for Risks and Failures of Biomaterials and Devices. J. Biomaterials Appl., 8, 64-71.

JAYABALAN M. and LIZYMOL P.P. (1997). Effect of autoclaving sterilization in the stability of polyurethane potting compounds based on caprolactone polyol. J. Polymer Material, 14, 49-55.

KAO W.J., HILTNER A., ANDERSON J.M. and LODOEN G.A. (1994). Theoretical analysis of in vivo macrophage adhesion and foreign body giant cell formation on strained poly(etherurethane urea) elastomers. J. Biomed. Mater. Res ., 28, 819-829.

KAROV J., CHAWLA A.S. and HINBERG I. (2000). Effect of reuse on surface characteristics of balloon angioplasty catheters. Artif. Cell. Blood Substit. Immobil. Biotechnol., 28, 229-240.

KATO S, MURO M, AKIFUSA S, HANADA N, SEMBA I, FUJI T, KOWASHI Y and NISHIHARA T. (1995). Evidence for apoptosis of murine macrophages by actinobacillus actinomycetemcomitans infection. Infection & Immunol., 63, 3914-3919.

KUDRYK V.L., SCHEIDT M.J., MCQUADE M.J., SUTHERLAND D.E., VANDYKE T.E. and HOLLINGER J.O. (1992). Toxic effect of ethylene-oxide-sterilized freeze-dried bone allograft on human gingival fibroblasts. J. Biomed. Mater. Res., 26, 1477-1488.

KYI M.S., HOLTON J. and RIDGWAY G.L. (1995). Assessment of the efficacy of a low temperature hydrogen peroxide gas plasma sterilization system. J. Hosp. Infect., 31, 275-284.

LABOW S. R, ERFLE J D and SANTERRE P. J. (1995). Neutrophil-mediated degradation of segmented polyurethanes. Biomaterials, 16, 51-59.

LABOW R.S., MEEK E. and SANTERRE J.P. (1999). Synthesis of cholesterol esterase by monocyte-derived macrophages: a potential role in the biodegradation of poly(urethane)s. J. Biomater.Appl., 13, 187-205.

LAMBA N. M. K., WOODHOUSE K. A. and COOPER S. L., (1998). Polyurethanes In Biomedical Application. CRC Press LLC, Boca Raton, FL. 205-241.

LELAH M. D. and COOPER S. L. (1986). Polyurethanes In Medicine. CRC Press, Boca Raton, FL., 22-33; 151-182.

LEROUGE S., GUIGNOT C., TABRIZIAN M., FERRIER D., YAGOUBI N. and YAHIA L'H. (2000). Plasma-based sterilization: Effct on surface and bulk properties and hydrolytic stability of reprocessed polyurethane electrophysiology catheters. J. Biomed. Mater. Res., 52, 774-782.

LIND P., DALENE M., SKARPINH G., qnd HAGMAR L. (1996). Toxicokinetics of 2,4- and 2,6- toluenediamine in hydrolysed urine and plasma after occupationa wxposure to 2,4- and 2,6- toluene diisocyanate. Occup. Environ. Med., 53, 94-99.

LISA SCOTT. (1995). Researchers test safety of medical device reuse. Modern Healthcare, 24, 78.

LUU H.M., BILES J. and WHITE K.D. (1994) Characterization of Plyesteruretahane Degradation Products. J. of Applied Biomaterials, 5, 1-7.

MARCHANT R., HILTNER A., HAMLIN C., RABINOVITCH A., SLOBODKIN R. and ANDERSON J.M. (1983). In vivo biocompatibility studies. The cage implant system and a biodegradable hydrogel. J. Biomed. Mater. Res., 17, 301-325.

MARCHANT R.E., ANDERSON J.M., HILTNER A., CASTILLO E.J., GLEIT J. and RATNER B.D. (1986). The biocompatibility of solution cast and acetone-extracted cast Biomer. J. Biomed. Mater. Res., 20, 799-815.

MARTZ H., PAYNTER R., BEN SLIMANE S., BEAUDOIN G., GUIDOIN R., BORZONE J., BEN SIMHON H., SATIN R. and SHEINER N. (1988). Hydrophilic microporous polyurethane versus expanded PTFE grafts as substitutes in the carotid arteries of dogs. A limited study. J. Biomed. Mater. Res. 22, 63-69.

MAURIN N., DATY N., GUERNIER C., DAHMEN K. and RICHTER H. (1997). An in vivo study of the biodegradation of the hydrophilic Mitrathane. J. Biomed. Mater. Res., 34, 73-78.

MAZZU A.L and SMITH C.P. (1984). Deterinimntiom of Extreactable Methylenedianiline in Thermoplastic Polyurethane by HPLC. J. Biomed. Matre Tes., 18, 961-968.

MCCARTHY S.J., MEIJS G.F., MITCHELL N., GUNATILLAKE P.A., HEATH G., BRANDWOOD A., and SCHINDHELM K. (1997). In-vivo degradation of polyurethanes: transmission-FTIR microscopic characterization of polyurethanes sectioned by cryomicrotomy. Biomaterials, 18, 1387-1409.

MEIJS G.F., MCCARTHY S.J., RIZZARDO E., CHEN Y.C. and CHATERLIER R.C. (1993). Degradation of mediacal-grad polyurethane elastomers: The effect of hydrogen peroxide *in vitro*. J. Biomed. Mater. Res., 27, 345-356.

MILLER K.M. and ANDERSON J.M. (1989). In vitro stimulation of fibroblast activity by factors generated from human monocytes activated by biomedical polymers. J. Biomed. Mater. Res., 23, 911-930.

MOHANTY M., HUNT J.A., DOHERTY P.J., ANNIS D. and WILLIAMS D.F. (1992). Evaluation of soft tissue response to a polyurethaneurea. Biomaterials, 13, 651-656.

MOREHEAD J.M. and HOLT G.R. (1994). Soft-tissue response to synthetic biomaterials. Otolaryngol Clin. North Am., 27, 195-201.

MAZZU A.L. and SMITH C.P. (1984). Determination of extractable methylene dianiline in thermoplastic polyurethanes by HPLC. J. Biomed. Mater. Res., 18, 961-968.

NAIR P. D. (1995). Currently practiced sterilization methods-Some inadvertent consequences. J. Biomater Appl., 10, 121-135.

NAKAOKA R., TSUCHIYA T. and NAKAMURA A. (2000). Studies on the mechanisms of tumorigenesis induced by polyetherurethane in rats: production of superoxide, tumor necrosis factor, and interleukin 1 from macrophages cultured on different polyetherurethanes. J. Biomed. Mater. Res., 49, 99-105.

NATIONAL TOXICOLOGY PROGRAM. Toxicology and carcinogenesis studies of ethylene oxide (CAS No. 75-21-8) in B6351mice (inhalation studies). Technical report series no 326. Research triangle Park, NC: U.S. Department of Health and Human Services. Public Health Service, National Institutes of Health. (NIH publication no.88-2582).

OKOGEL T., SOLDANI G., GODDARD M. and GALLETTI P.M. (1996). Penetrating micropores increase patency and achieve extensive endothelialization in

small diameter polymer skin coated vascular grafts. Trans. Am. Soc. Artif. Intern Organs., 42, M398-401.

PANICHI V., MIGLIORI M., DE PIETRO S., TACCOLA D., ANDREINI B., METELLI M.R., GIOVANNINI L. and PALLA R. (2000). The link of biocompatibility to cytokine production. Kidney Int., 58 Suppl, 76, S96-103.

PARK J.H., PARK K.D., and BAE Y.H. (1999). PDMS-based polyurethanes with MPEG grafts: sythesis, characterization and platelet adhesion study. Biomaterials. 20, 943-953.

PATEL T., GORES G.J. and KAUFMANN S.H. The role of proteases during apoptosis. (1996). FASEB J., 10, 587-597.

PAULUHN J. (2000). Acute inhalation toxicity of polymeric diphenyl-methane 4,4'-diisocyanate in rats: time course of changes in bronchoalveolar lavage. Arch. Toxicol., 74, 257-269.

PAYNTER R.W., ASKILL I.N., GLICK S.H., and GUIDOIN R. (1988). The hydrolytic stability of Mitrathane (a polyurethane urea)--an x-ray photoelectron spectroscopy study. J. Biomed. Mater. Res., 22, 687-698.

PENNA T.C. and FERRAZ C.A. (2000). Cleaning of blood-contaminated reprocessed angiographic catheters and spinal needles. Infect. Control Hosp. Epidemiol., 21, 499-504.

PHILLIPS R, FREY M. and MARTIN R. O. (1986). Long-term performance of polyurethane pacing leads: mechanisms of design-related failures. Pacing. Clin. Electrophysiol., 9, 1166-1172

PICHA G.J., GOLDSTEIN J.A. and STOHR E. (1990). Natural-Y Meme polyurethane versus smooth silicone: analysis of the soft-tissue interaction from 3 days to 1 year in the rat animal model. Plast. Reconstr. Surg., 85, 903-916.

PINCHUK L. (1994). A review of the biostability and carcinogenicity of polyurethanes in medicine and the new generation of 'biostable' polyurethanes. J. Biomater. Sci. Polym. Ed., 6, 225-267.

PIZZOFERRATO A., ARCIOLA C.R., CENNI E., CIAPETTI G. and SASI S. (1995). In vitro biocompatibility of a polyurethane catheter after deposition of fluorinated film. Biomaterials, 16, 361-367.

REHMAN I.U. (1996). Biodegradable polyurethanes: biodegradable low adherence films for the prevention of adhesions after surgery. J. Biomater. Appl., 11, 182-257.

REPORT FROM THE CANADIAN COORDINATING OFFICE FOR HEALTH TECHNOLOGY. (1991). Assessment (CCOHTA). Reuse of single-use cardiac catheters. Int. J. Technol. Assess. Health Care, 7, 637-638.

RICE J. M., FISHER A. C and HUNT J. A. (1998). Macrophage-polymer interaction. J. Biomater. Science, 9, 833-847.

RUTALA W.A., GERGEN M.F. and WEBER D.J. (1998). Comparative evaluation of the sporicidal activity of new low-temperature sterilization technologies: ethylene oxide, 2 plasma sterilization systems, and liquid peracetic acid. Am. J. Infect. Control., 26, 393-398.

SAAD B., MATTER S., CIARDELLI G., UHLSCHMID G.K., WELTI M., NEUENSCHWANDER P. and SUTER U.W. (1996). Interactions of osteoblasts and macrophages with biodegradable and highly porous polyesterurethane foam and its degradation products. J. Biomed. Mater. Res., 32, 355-366.

SAAD B., HIRT T.D., WELTI M., UHLSCHMID G.K., NEUENSCHWANDER P. and SUTER U.W. (1997). Development of degradable polyesterurethanes for medical applications: in vitro and in vivo evaluations. J. Biomed. Mater. Res., 36, 65-74.

SAAD B. MATTERG., WELTI M., UHLSCHMID G. K., NEUENSCHWANDER P. and SUTER U. W. (1998). Degradable and highly porous polyesterurethane foam as biomateerial: Effects and phagocytosis of degredation products in osteoblasts. J. Biomed. Mater. Res., 39, 594-602.

SANTERRE J.P., LABOW R.S., DUGUAY D.G., ERFLE D., and ADAMS G.A. (1994). Biodegradation evaluation of polyether and polyester-urethanes with oxidative and hydrolytic enzymes. J Biomed Mater Res., 28, 1187-1199.

SATO M., XI T.F., NAKSMURA A., KAWASAKI Y., UMEMURE T., TSUDA M. and KUROKAWA Y. (1995). Degradation of polyetherurethane by subcutaneous implantation into rats. II. Changes of contact angles infrared spectra, and nuclear magnetic resonance spectra. J. Biomed. Mater. Res., 29, 1201-1213.

SHNTANI H. (1995). Formation and elution of toxic compounds from sterilized medical products: methylenedianiline formation in polyurethane. J. Bioma. Appli., 10, 23-36.

SINCLAIR T. M, KERRIGAN C. L, and SAMPALIS J. (1995). Biodegradation of polyurethane foam, revisited, in the rat model. Plast. Reconstr. Surg., 9, 1326-1335.

STOKES K. and COBIAN K. (1982). Polyether polyurethanes for implantable pacemaker leads. Biomaterials, 3, 225.

STOKES KB, CHURCH T. (1986). Ten-year experience with implanted polyurethane lead insulation. Pacing Clin. Electrophysiol., 9, 1160-1165.

STOKES K., COURY A. and URBANSKI P. (1987). Autooxidative degradation of implanted polyether polyurethane devices. J. Biomater. Appl. 1,411-448.

STOKE K. and MCVENES R. (1995). Polyurethane Elastomer Biostability. J. Biomater. Appl., 9, 321-355.

SZYCHER M. and MCARTHUR W. A. (1985). Surface Fissuring of Polyurethanes Following *in vivo* Exposure. Corrosion and Degradation of Implant Materials. ASTM STP 859, Fraker and Griffin, eds., Philadelphia, PA, 308-321.

SZYCHER M. (1991). High performance biomaterials. Technomic Publishing Inc., Lancaster, Pennsylvania, USA, 233-244.

SZYCHER M., SICILIANO A. A. and Reed A.M. (1991). Polyurethanes in medical devices. Med. Res. Mater., 1, 18-25.

SZYCHER M., REED A. M. and SICILIANO A. A. (1991). *In vivo* testing of a biostable polyurethane. J. Biomater. Appl., 6, 110-130.

SZYCHER M. and REED A.M. (1992). Biostable polyurethane elastomers. Med. Device Technol., 3, 42-51.

TABATA Y. and IKADA Y. (1988). Effect of the size and surface charge of polymer microspheres on their phagocytosis by macrophage. Biomaterials, 9, 356-362.

TSUCHIYA T., TAKAHARA A., COOPER S.L. and NAKAMURA A. (1995). Studies on the tumor-promoting activity of polyurethanes: depletion of inhibitory action of metabolic cooperation on the surface of a polyalkyleneurethane but not a polyetherurethane. J. Biomed. Mater. Res., 29,835-841.

TABRIZIAN M., LEROUGE S., WERTHEIMER M.R., MARCHAND R. and L'HOCINE Y. (1997). Surface modification of polymer-based devices by cold sterilization techniques. Proceedings of the First International Symposium on Advanced Biomaterials. Montréal, Québec, Canada.

TANG L. and EATON J.W. (1995). Inflammatory responses to biomaterials. Am. J. Clin. Pathol., 103, 466-471.

TANG L., UGAROVA T.P., PLOW E.F. and EATON J.W. (1996). Molecular determinants of acute inflammatory responses to biomaterials. J. Clin. Invest., 97, 1329-1334.

TANG Y. W., SANTERRE J. P., LABOW R. S. and TAYLOR D.G. (1997). Application of macromolecular additives to reduce the hydrolytic degradation of polyurethanes by lysosomal enzymes. Biomaterials,18, 37-45.

TRINDADE M.C., LIND M., GOODMAN S.B., MALONEY W.J., SCHURMAN D.J. and SMITH RL. (1999). Interferon-gamma exacerbates polymethylmethacrylate particle-induced interleukin-6 release by human monocyte/macrophages in vitro. J. Biomed. Mater. Res., 47, 1-7.

TSENG Y.C., TABATA Y., HYON S.H. and IKADA Y. (1990). In vitro toxicity test of 2-cyanoacrylate polymers by cell culture method. J. Biomed. Mater. Res., 24, 1355-1367.

TSUCHIYA T., NAKAOKA R., DEGAWA H. and NAKAMURA A. (1996). Studies on the mechanisms of tumorigenesis induced by polyetherurethanes in rats: leachable and

biodegradable oligomers involving the diphenyl carbamate structure acted as an initiator on the transformation of Balb 3T3 cells. J. Biomed. Mater. Res., 31, 299-303.

TYLER B. J. and RATNER B. D. (1994). Oxidative degradation of Biomer fractions prepared by using preparative-scale gel permeation chromatography. J. Biomater. Sci. Polym., 6, 359-373.

VAN DER GIESSEN W.J., LINCOFF A.M., SCHWARTZ R.S., VAN BEUSEKOM H.M., SERRUYS P.W., HOLMES DR J.R., ELLIS S.G. and TOPOL E.J. (1996). Marked inflammatory sequelae to implantation of biodegradable and nonbiodegradable polymers in porcine coronary arteries. Circulation, 94, 1690-1697.

VAN DER LEI B., NIEUWENHUIS P., MOLENAAR I. and WILDEVUUR C.R. (1987). Long-term biologic fate of neoarteries regenerated in microporous, compliant, biodegradable, small-caliber vascular grafts in rats. Surgery, 101, 459-467.

VEZEAU P. J., KOORBUSCH G. F., DRAUGHN R. A. and KELLER J. C. (1996). Effect of multiple sterilization on surface characteristics and in vitro biologic responses to titanium. J. Oral Maxilloface Surg., 54, 738-746.

VERMETTE P., WANG G.B., SANTERRE J.P., THIBAULT J. and LAROCHE G. (1999). Commercial polyurethanes: the potential influence of auxiliary chemicals on the biodegradation process. J. Biomater. Sci. Plym., 10, 729-749.

VINCE D.G, HUNT J.A. and WILLIAMS D.F. (1991). Quantitative assessment of the tissue response to implanted biomaterials. Biomaterials, 12, 731-736.

VINK P. and PLEIJSIER K. (1986). Aeration of ethylene oxide-sterilized polymers. Biomaterials, 7, 225-230.

WANG B. M., CHANG B. W., Sargeant R. and Manson P.N. (1998). Late capsular hematoma after breast reconstruction with polyurethane-covered implants. Plastic. & Recinstructive Surgery, 102: 450-452.

WANG G.B., SANTERRE J.P. and LABOW R.S. (1997). High-performance liquid chromatographic separation and tandem mass spectrometric identification of breakdown products associated with the biological hydrolysis of a biomedical polyurethane. J. Chromatogr. B. Biomed. Sci. Appl., 26, 69-80.

WEBER D. J. AND RUTALA W. A. (1998). Occupational risks associated with the use of selected disinfectants and steriliants. In: Rutala W. A. ed. Disinfection, Sterilization and antisepsis in Health Care. Champlain, NY: Polyscience Publications. 211-226.

WIGGINS M. J., WILKOFF B., ANDERSON J. M., and HILTNER A. (2001). Biodegradation of polyether polyurethane inner insulation in bipolar pacemaker leads. J. Biomed. Mater. Res., 58, 302-307.

WILLIAMS D.F. (1991). Biocompatibility, an overview. In the Concise Encyclopedia of Medical and Dental Matarials Ed. Williams D.F. Pergamon Press, Oxford, UK.

WOO M., HAKEM R., SOENGAS M.S., DUNCAN G.S., SHAHINIAN A., KAGI D., HAKEM A., MCCURRACH M., KHOO W., KAUFMAN S.A., SENALDI G., HOWARD T., LOWE S.W. and MAK T.W. (1998). Essential contribution of caspase 3/CPP32 to apoptosis and its associated nuclear changes. Genes Dev., 15, 806-819.

WU Y., ZHAO Q., ANDERSON J.M., HILTNER A., LODOEN G.A. and PAYET C.R. (1991). Effect of some additives on the biostability of a poly(etherurethane) elastomer. J. Biomed. Mater. Res., 25, 1415-1416.

XI T., SATO M., NAKAMURA A., KAWASAKI Y., UMEMURA T., TSUDA M. and KUROKAWA Y. (1994). Degradation of polyetherurethane by subcutaneous implantation into rats. I. Molecular weight change and surface morphology. J. Biomed. Mater. Res., 28, 483-490.

ZDRAHALA R. J. (1996). Small carliber vascular grafts. Part II: Polyurethanes revisited. J. Biomater. Appl., 1, 37-61.

ZHANG Z., KING M., GUIDOIN R., THERRIEN M., DOILLON C., DIEHL-JONES W.L. and HUEBNER E. (1994). In vitro exposure of a novel polyesterurethane graft to enzymes: a study of the biostability of the Vascugraft arterial prosthesis. Biomaterials, 15, 1129-1144.

ZHANG Y. Z, BJURSTEN L.M, FREIJ-LARSSON C., KOBER M. and WESSLEN B. (1996). Tissue response to commercial silicone and polyurethane elastomers after different sterilization procedures. Biomaterials, 17, 2265-2272.

ZHANG Z, MAROIS Y, GUIDOIN R.G., BULL P., MAROIS M., HOW T., LAROCHE G. and KING M.W. (1997). Vascugraft polyurethane arterial prosthesis as femoro-popliteal and femoro-peroneal bypasses in humans: pathological, structural and chemical analyses of four excised grafts. Biomaterials, 18, 113-124.

ZHAO Q, AGGER M. P., FITZPATRICK M., ANDERSON J. M., HILTNER A., STOKES K. and URBANSKI P. (1990). Cellular interactions with biomaterials: in vivo cracking of pre-stressed Pellethane 2363-80A. J. Biomed. Mater. Res., 24, 621-637.

ZHAO Q.H., MCNALLY A.K., RUBIN K.R., RENIER M., WU Y., ROSE-CAPRARA V., ANDERSON J.M., HILTNER A., URBANSKI P. and STOKES K. (1993). Human plasma alpha 2-macroglobulin promotes in vitro oxidative stress cracking of Pellethane 2363-80A: in vivo and in vitro correlation. J. Biomed. Mater. Res., 27, 379-388.