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Rhinology

In vivo tissue response and durability of five novel synthetic polymers in a rabbit model

Biocompatibilità e durata in vivo di cinque nuovi polimeri sintetici testati su coniglio

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SUMMARY

Alloplastic materials are frequently used in facial plastic surgeries such as rhinoplasty and nasal reconstruction. Unfortunately, the ideal alloplastic material has not been found. This experimental study evaluates the tissue response and durability of five novel polymers developed as an alloplastic material. In this experimental study involving a tertiary university hospital, six subcuticular pockets were formed at the back of 10 rabbits for the implantation of each polymer and sham group. Each pocket was excised with its adjacent tissue after three months, and collected for histopathological examination. Semi-quantitative examination including neovascularisation, inflammation, fibrosis, abscess formation, multinucleated foreign body giant cells was performed, and integrity of polymer was evaluated. A statistical comparison was performed. No statically significant difference was detected in neovascularisation, inflammation, fibrosis, abscess formation and multinucleated foreign body giant cells when a paired comparison between sham and polymer II, III and IV groups was performed individually. Nevertheless, the degree of fibrosis was less than sham group in polymer I (p = .027) and V (p = .018), although the other variables were almost similar. The integrity of polymers III (9 intact, 1 fragmented) and IV (8 intact, 2 absent) was better than the other polymers. These novel synthetic polymers could be considered as good candidates for clinical applicability. All polymers provided satisfactory results in terms of tissue response; however, fibrovascular integration was higher in polymers II, III and IV. In addition, the durability of polymer III and IV was better than the others.

KEY WORDS: Alloplastic material • Polymer • Rhinoplasty • Nasal reconstruction • Bioavailability

RIASSUNTO

I materiali alloplastici vengono frequentemente utilizzati negli interventi di chirurgia plastica sul volto, quali la rinoplastica e la chirurgia ricostruttiva del naso. Ad oggi non è stato ancora individuato un materiale alloplastico con caratteristiche ottimali. Il presente studio sperimentale si propone di valutare la risposta tissutale e la resistenza nel tempo di cinque nuovi polimeri proposti come materiali alloplastici. Il presente studio è stato condotto presso un ospedale universitario di terzo livello. Sono state ricavate sei tasche sottocutanee sul dorso di 10 conigli che sono state usate per l'impianto di ciascuno dei polimeri testati più una tasca di controllo. Ciascuna delle tasche è stata escissa congiuntamente al tessuto circostante dopo tre mesi, ed è stata sottoposta ad un esame istopatologico. È stata quindi condotta una valutazione semi quantitativa con focus su neo angiogenesi, infiammazione, fibrosi, formazione di ascessi, presenza di cellule giganti multinucleate contenenti corpi estranei e stato dei polimeri testati. E' stata inoltre effettuata una valutazione statistica, che per quanto riguarda la comparazione diretta fra la tasca di controllo e i polimeri II, III e IV non ha mostrato differenze significative in merito alla neo vascolarizzazione, all'infiammazione, alla fibrosi, alla presenza di ascessi ed alla presenza di cellule giganti multinucleate. Il polimero I ha invece mostrato un grado di fibrosi inferiore rispetto alla tasca di controllo (p = .027) and V (p = .018), benché le altre variabili prese in considerazione fossero sostanzialmente uguali. L'integrità nel tempo dei polimeri III (9 intatti, uno frammentato) e IV (8 intatti, 2 assenti) è stata migliore di quella ottenuta con gli altri polimeri testati. Questo gruppo di nuovi polimeri può essere considerato interessante per future applicazioni cliniche. Tutti i polimeri hanno mostrato risultati accettabili in termini di risposta dei tessuti, tuttavia i fenomeni di integrazione fibrovascolare sono stati maggiori nel caso dei polimeri II, III e IV. Inoltre la durata nel tempo dei polimeri III e IV è stata la migliore in assoluto.

PAROLE CHIAVE: *Materiali alloplastici • Polimeri • Rinoplastica • Ricostruzione nasale • Biocompatibilità*

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Introduction

Alloplastic materials play a significant role and are widely used in the field of facial plastic and reconstructive surgery, although autogenous tissues (e.g. cartilage, bone, skin/dermis, etc.) are generally preferred for most cases, if possible. They generally provide a significant decrease in operative time and prevent donor-site morbidity, especially in revision cases in whom a second surgical site for harvesting a graft is almost always inevitable [1]. A virtual explosion in the technologies of alloplastic materials has taken place; indeed, several types of different alloplastic materials such as expanded polytetrafluoroethylene (Gore-Tex; W. L. Gore and Associates, Flagstaff,Ariz), silicone rubber (such as silastic), polyethylene (such as Medpore; Porex, Fairburn, Ga), plastipore (Richards Manufacturing Company, Memphis, Tenn), polyesters and polyamides (such as Dacron; Ethicon Inc., Somerville, NJ), Mersilene (Ethicon Inc), Supramid (S. Jackson Inc, Alexandria, Va), Cooley Dacron knitted implant (Meadox; Boston Scientific, Quincy, Mass) have been used in different aspects of surgery in order to reconstruct or augment facial structures or improve deformities. Unfortunately, most of these alloplastic materials have different amounts of potential risk for inflammatory reaction, extrusion, infection and resorption [2-5]. Therefore, an ideal alloplastic material should be: (i) biocompatible, (ii) non-carcinogenic, (iii) non-mutagenic, (iv) non-antigenic, (v) resistant to infections, (vi) durable, (vii) easily carved, (viii) pliable, (ix) easily fixed and removed, (x) inexpensive and (xi) available in sufficient quantities.

Biocompatibility has been recently defined as "*the ability of a material to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy*"[6]. Therefore, the main component of biocompatibility is tissue response. It is well known that once a tissue is injured for the implantation of an alloplastic material, a wound healing response which constitutes a sequence of complex events such as neovascularisation, inflammatory reaction, fibrosis and foreign body reaction take place in the adjacent tissues. Experimental and clinical studies have demonstrated that physical and chemical properties of alloplastic materials may influence and affect host response and lead to extrusion, overinflammation, infection and resorption or fragmentation of implants [2-5]. Therefore, the search for an ideal alloplastic material still remains a challenge. In this study, five novel synthetic polymers were introduced as potential candidates for clinical application. Moreover, durability of polymers and quality and intensity of tissue response against polymers were histologically examined in a rabbit model.

Materials and methods

The experimental study was approved by the Research Ethics Committee of the Eskişehir Osmangazi University and DETAM (Eskişehir Osmangazi University Hospital Experimental Study Center), Eskişehir. All procedures were supervised by a veterinarian. Animals were placed in appropriate cages and had free access to water and a standardised commercial ration.

Ten adult New Zealand Albino rabbits, weighting between 2.5 and 4 kg and aged between 15 to 18 months, were included and followed for three months. The pieces of polymers were prepared in a standardised fashion (0.5x0.5 cm in size). All pieces were packed separately and sterilised in a gas autoclave prior to surgery.

Polymer production

Five newly synthesised polymers were used in this study. The properties of these materials were as follows:

1. ELASTOSIL LR3003/20 (shore hardness 30A, soft);
2. ELASTOSIL LR3003/30 (shore hardness 37A, medium soft);
3. IY-PO-03-149-B FTPU (fluorinated thermoplastic polyurethane) (shore hardness 50A, medium soft);
4. PTMO-1K/PDMS/50% EXTR (shore hardness 90A, hard);
5. PTMO1K/PDMS/40% EXTR (shore hardness 80A, hard).

Physical and chemical properties of polymers

ELASTOSIL LR3003/20 and ELASTOSIL LR3003/30: Both polymers were highly elastic, cross-linked silicone rubbers supplied by Wacker Chemie. They were obtained by the platinum catalysed reactions of methylhydrogensiloxane oligomers with methylvinylsiloxane oligomers. Elastosil rubbers were usually filled with small amounts of fumed silica and display good mechanical integrity.

IY-PU-03-149-B (FTPU (fluorinated thermoplastic polyurethane): Poly(tetramethylene oxide) glycol (PTMO-2000) with a <Mn> value of 2040 g/mol was kindly provided by DuPont, USA. Fluorolink E10 H, which is an ethylene glycol terminated perfluoroether oligomer (E10 H) with a <Mn> value of 1400 g/mol, is a product of Solvay Solexis, Belgium. Bis(4-isocyanatocyclohexyl) methane (HMDI) (99.5%) was supplied by Bayer. 2-methyl-1,5-diaminopentane (Dytek A) (DuPont) and reagent grade reaction solvents, isopropyl alcohol (IPA) (Merck) and tetrahydrofuran (THF) (Merck) were all used as received. Dibutyltindilaurate (DBTDL) catalyst was obtained from Air Products, USA. The polymerisation procedure was conducted in a 3-neck, round-bottom Pyrex flask equipped with an overhead stirrer, addition funnel and thermometer. Reaction was carried out by using a two-step procedure. PTMO-2000 2.283 g (1.119 mmol), E10 H 2.264 g (1.617 mmol) and HMDI 1.203 g (4.585

mmol) were weighed into the reactor, stirred and heated to 70^{0}C. Next, 150 ppm of DBTDL in THF was added as the catalyst and the reaction was continued for 60 min to form the prepolymer. The mixture was then cooled to room temperature, dissolved in 15 g of THF and diluted with 8 g of IPA. Chain extender, 0.215 g (1.849 mmol) Dytek A, was dissolved in 7 g IPA and added to the reaction mixture drop-wise, under strong agitation. The yield was quantitative. Polymer films were prepared by solution casting into Teflon molds from THF/IPA. The solvent was first evaporated in an air oven at 50^{0}C overnight and then in a vacuum oven at 50^{0}C until constant weight was reached. Films obtained were kept in sealed polyethylene bags in a desiccator.

PTMO-1K/PDMS/40% EXTR and PTMO1K/PDMS/50% EXTR: These polymers are polyurethaneurea elastomers based on PTMO-1000 and polydimethylsiloxane (PDMS-2000). They contained 40% and 50% by weight of PDMS, respectively, for improved biocompatibility. They were obtained by melt polymerisation in a twin-screw extruder.

Animals and implantation procedure

All experiments were performed under anaesthesia using intramuscular injection of xylazine (5 mg/kg) and ketamine (50 mg/kg). Six surgical pockets for five polymer implantations and sham operation were generated to the dorsal area of rabbits after a skin incision of 1.5 cm in size, and undermining and elevation of subcutaneous tissue. All surgical pockets were performed approximately 2 cm apart from each other. Afterwards, pieces of preshaped and sterilised polymers were administered into the surgical pockets and placed just over muscles, under the subcutaneous tissue. Skin incisions were closed by simple interrupted sutures of mononylon 3-0 sutures. In the sham group, all surgical procedures were performed similarly except for polymer implantation.

The animals were given a single injection of intramuscular ceftriaxone (100 mg/kg) for five days, and followed for a period of three months. None of the polymers were extruded during the experimental period. All rabbits were sacrificed with anaesthetic (combination of xylazine and ketamine) overdose at the end of 3 months. The surgical pockets at the sites of polymer implantation and sham sites were dissected and excised. The integrity of polymer (absent, fragmented or intact) was evaluated and noted initially. Finally, all specimens were immediately fixed in neutralised 10% buffered formaldehyde for histopathological examination.

Histopathological examination

Sections of 5 μm in size were obtained from paraffin blocks and processed individually. Paraffin sections were submitted to deparaffinisation in xylene for a short time and followed by rehydration in decreasing alcohol solutions. All sections were embedded. The paraffin sections were stained with haematoxylin-eosin and toluidine Blue for histopathological examination. The areas of tissue adjacent to the implants were first observed under low magnification and later scrutinised under high magnification. The tissue response was examined and graded with the following criteria: (i) vascular congestion (mild congestion, significant congestion with dilated vessels, highly dilated vessels with red blood cell extravasation), (ii) inflammation (absent, mild, moderate, intense), (iii) fibrosis (absent, present of fibroblasts alone, reparative fibroblastic proliferation with thickness), (iv) abscess formation (absent, present), (v) foreign body giant cell (absent, present).

All histopathological examinations were performed by a blinded board-certified pathologist.

Statistical analysis

The statistical analysis was performed using SPSS for Windows 17.0. A paired comparison between polymers and sham group was performed for each variable individually using the chi square test. A p value <0.05 was considered statistically significant.

Results

None of the animals was lost before the planned schedule. All polymers were tolerated well without causing gross infection, and no extrusion was observed. The distribution of tissue responses in polymer and sham groups are presented in Figure 1. The degree of vascularisation is very similar to sham group in polymers II and III, although none of the polymers demonstrated a statistically significant difference compared with the sham group (Table I). The inflammatory reaction against all polymers was comparable with sham group (Fig. 2A-C, Table I). No significant difference was observed when comparisons between polymer II-sham group, polymer III-sham group and polymer IV-sham group were performed according to the degree of fibrosis (Fig. 2A-C, Table I). On the other hand, the statistical comparison between polymer I-sham group and polymer V-sham group showed significant differences in the favour of sham group ($p = .027$ and $p = .018$). Finally, when polymer groups were individually compared with sham group according to the presence of abscess formation and multinucleated foreign body giant cell, none of the polymers showed a statistically significant difference (Table I).

The durability of polymer III was considered excellent (90% intact) as shown in Figure 1. In addition, the durability of polymer IV (80% intact) and I (70% intact) was acceptable. However, more than half of polymer II was fragmented or lost at the end of the experiment. However, no significant difference was detected when polymer II-III, polymer II-IV, polymer II-I and polymer II-V were

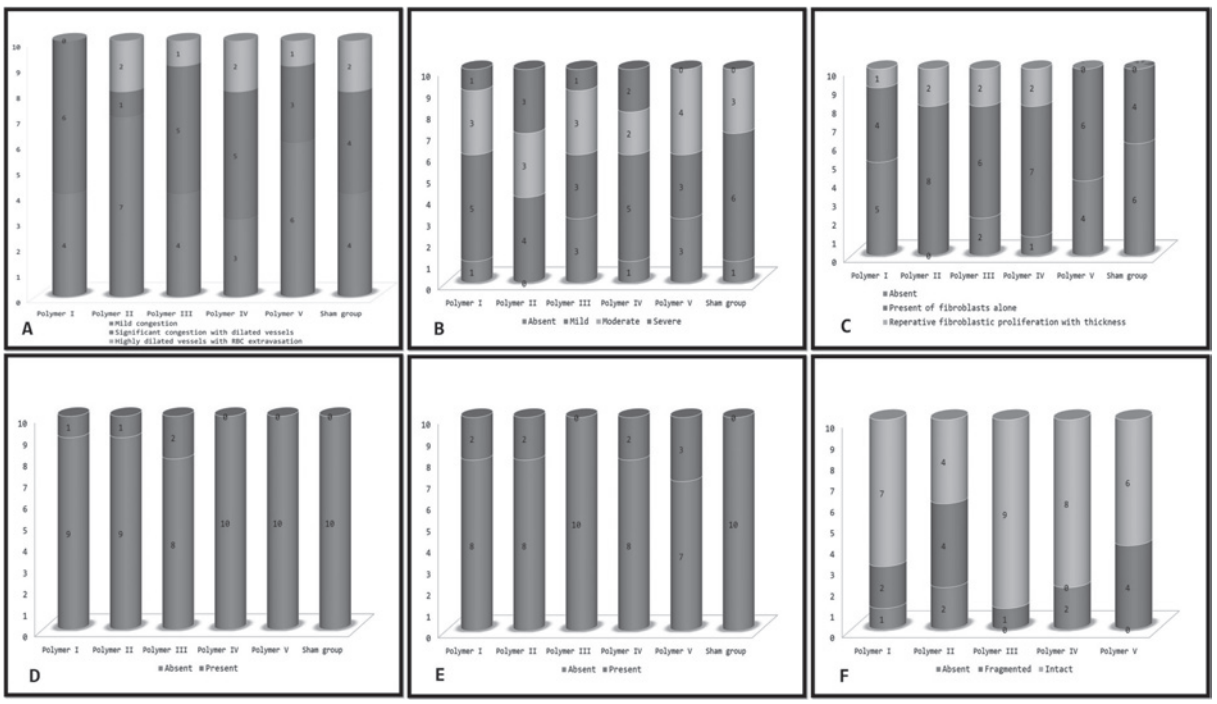

Fig. 1. The distribution of tissue response [neovascularisation (A), inflammation (B), fibrosis (C), abscess formation (D), foreign body giant cell (E) and integrity of polymers (F)] in polymer and sham groups.

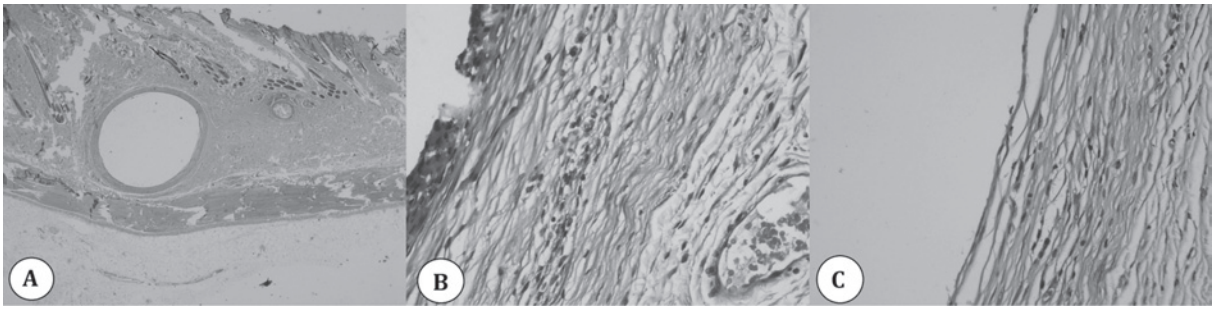

Fig. 2. Photomicrograph showing the intact implant material (haemotoxylin and eosin, x40). (A), Infiltration of the connective tissue capsule surrounding the implant by the inflammatory infiltrate which is mainly composed of lymphocytes and plasma cells (B), accompanied by eosinophils in some (C).

compared according to durability ($p = 0.057$, $p = 0.069$, $p = 0.403$ and $p = 0.301$).

Discussion

Several alloplastic materials have been used in surgery. In general, they shorten the duration of surgery, reduce trauma to donor region and are readily available. However, one of the main drawbacks of these alloplastic materials is tissue response, which may also lead to extrusion and/or poor resistance to infection. Therefore, the quest for finding an ideal alloplastic material still remains of wide interest. In this study, five new synthetic polymers, differing in physical structure and hardness, were introduced as potential candidates for clinical application. An *in vitro* experimental model was preferred for assessment of tissue response and durability of these polymers, and histopathological examination, a gold standard technique

Table I. Statistical comparison between polymer and sham groups according to neovascularisation, inflammation, fibrosis, abscess formation and multinucleated foreign body giant cells.

	Neovascularisation	Inflammation	Fibrosis	Abscess formation	Multinucleated foreign body giant cell
Polymer I-Sham group	0.301	0.779	0.027†	0.305	0.136
Polymer I-Polymer II	0.041†	.550	0.036†	1.000	1.000
Polymer I-Polymer III	0.580	0.682	0.364	0.531	0.136
Polymer I-Polymer IV	0.327	0.912	0.148	0.305	1.000
Polymer I-Polymer V	0.301	0.450	0.470	0.305	0.606
Polymer II-Sham group	0.270	0.221	0.329	0.305	0.136
Polymer II-Polymer III	0.148	0.246	0.319	0.531	0.136
Polymer II-Polymer IV	0.118	0.680	0.587	0.305	1.000
Polymer II-Polymer V	0.494	0.099	0.043†	0.305	0.606
Polymer III-Sham group	0.801	0.392	0.264	0.136	NS
Polymer III-Polymer IV	0.788	0.566	0.815	0.136	0.136
Polymer III-Polymer V	0.638	0.767	0.264	0.136	0.060
Polymer IV-Sham group	0.881	0.514	0.418	NS	0.136
Polymer IV-Polymer V	0.400	0.244	0.144	NS	0.606
Polymer V-Sham group	0.645	0.343	0.018	NS	0.060

NS: Not computed because parameter was a constant. †Statistically significant ($p<0.05$).

for determining the degree of tissue response, was performed [3 5 7]. The assessment of tissue response includes neovascularisation, inflammation, fibrosis, abscess formation and multinucleated foreign body giant cells.

Previous histopathological examination of explanted porous polyethylene implants demonstrated a significant decrease in fibrovascular invasion, increase in inflammatory reaction and presence of multinucleated foreign body cells[8]. In this study, none of the polymers was extruded, which considered a high tissue ingrowth and low inflammatory response. In fact, implants that have a high capacity of fibrovascular integration are prone to behave more like natural tissue; thus, they can become more stable and more resistant to infections. In addition, Naik et al. emphasised the positive effect of vascularisation for reduction of extrusion, migration and infection after polymer implantation[9]. In this experimental study, assessment of neovascularisation showed similar histological findings in all experimental groups (polymer implanted and sham groups) (Fig. 1 and Table I). On the other hand, the degree of fibrosis seems in favour of polymers II, III and IV, although no statistically significant difference was detected when compared with the sham group (Fig. 1 and Table I). However, the degree of fibrosis was less than sham group for polymer I ($p = 0.027$) and V ($p = 0.018$). Therefore, complete invasion by fibrovascular tissue at the site of polymer implantation, especially in polymers II, III and IV, was demonstrated. Sclafani et al. examined the tolerability of porous high-density polyethylene and nonporous silicone implants in an experimental study, and observed better fibrovascular integration with porous high-density polyethylene[2]. Moreover, they detected no inflammatory cells in the periphery of implant, even though several other studies have demonstrated a vibrant inflammatory response with porous polytetrafluoroethylene [10-12]. This experimental study found no sign of increase in inflammatory response at the adjacent sites of polymer implantation (Fig. 1 and Table I). Moreover, the presence of multinucleated foreign body giant cell, an important indicator of vigorous inflammatory response to implants, was not significantly different when individual comparison between polymer and sham groups was performed (Table I). Therefore, high tolerability against all polymers was seen, although better results were observed with polymers III and IV.

The moulding and fashioning of an implant is crucial, especially for facial reconstructive and aesthetic surgeries. Softer implants are generally preferred because they can be easily carved and structured, and have a more natural appearance. Polymers I, II and III are softer materials; therefore, moulding and fashioning of these polymers is easier than with polymers IV and V. Finally, one of the most important characteristics of an ideal alloplastic material is the durability and/or firmness of an implant. An ideal implant should preserve its integrity, which is essential for long-term stability. In this experimental study, the durability of all synthetic polymers was acceptable, and no significant difference was seen when a paired comparison was performed (Fig. 1 and Table I). Nevertheless, polymers III (90% intact) and IV (80% intact) demonstrated the highest reliability.

Conclusions

Five novel synthetic polymers have been developed and introduced as potential candidates for clinical application. In this experimental study, histopathological examination of tissue response against these polymers demonstrated high tolerability, especially with polymers II, III and IV. In addition, polymers III and IV had a better durability and protected their integrity. Therefore, these synthetic polymers may be suitable for facial plastic and reconstructive surgery; however, further studies are also required to evaluate their biocompatibility.

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