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The small volume subcutaneous polyalkylimide injection-induced collagen capsule in rats: A preliminary study

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ABSTRACT

Background: Polyalkylimide is a permanent dermal filler that has been used for skin augmentation to ameliorate skin ageing. The massive volume injection has a higher risk of complication. This study aims to find collagen capsule formation by small volume polyalkylimide implantation.
Methods: We used an experimental post-test only control group design. The control group received subcutaneous NaCl injection, and the treatment group received subcutaneous polyalkylimide injection 0.018 mL (equal 1 mL in the humans). After five weeks, all rats (Rattus norvegicus) strain Wistar were sacrificed. The skin samples were collected and proceed for histologic staining. Capsule thickness was performed as mean ± SD.
Results: The treatment group showed polyalkylimide mass in subcutaneous tissue, collagen fibres surrounding the polyalkylimide, and no inflammatory cells. The collagen capsule thickness was 7.45 ± 0.78 µm.
Conclusion: The small volume polyalkylimide subcutaneous injection-induced collagen capsule formation. The small volume implantation shows promising effects for skin augmentation.

Keywords: *polyalkylimide, dermal filler, collagen capsule*
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INTRODUCTION

Skin is the largest organ when becoming ageing manifest as dry and thin skin, wrinkle, gradual atrophy, coarse skin, hyperpigmentation, laxity, sagging. Extracellular matrix and growth factors reduce in aged human skin. The skin ageing process causes skin atrophy. The skin loses its elasticity because of extracellular matrix degradation, especially collagen.[1] To improve skin ageing, it needs skin augmentation. The dermal filler is a non-invasive method for skin augmentation. The dermal filler improves skin atrophy by increasing skin volume and inducing collagen formation. The dermal filler as a foreign body has a mechanical stretching effect into soft tissue and causes foreign body reaction which stimulates growth factors and fibroblast proliferation.[2]

Polyalkylimide is a permanent filler that is ideal because of non-toxic, non-carcinogenic, non-allergenic, non-immunogenic, non-pyrogenic, non-migration, inert, long life span, and used as endoprosthesis in human since the year 2000. Polyalkylimide is a polymer compound that consists of 4% polymer polyalkylimide and 96% free pyrogenic water (pH 6.8-7.2). Polyalkylimide induces neocollagenesis and improves skin appearance. The present applications use the volume of 1.5-110 mL.[3] The large volume of implantation has a higher risk of complication in acute and chronic reactions. A study by Schelke et al., 2009, reported the patient complication rate was 4.8%, and treatment complication rate was 3.3%. The complications were inflammation, hardening, migration and accumulation of the product. The most common was inflammation.[4] Sattler et al., 2013, reported the complications of large volume polyalkylimide for pectus excavatum deformity. The patients received 10-340 ml, and the overall complication rate was 34.7%. The complications were swelling, inflammation, and infection.[5] All permanent fillers have common significant risks, namely over injection, granuloma formation, and migration. Granuloma is the manifestation of chronic reaction that develops after years in 1-3% of patients.6 This study aims to find the collagen capsule formation induced by small volume polyalkylimide (less than 1.5 mL). If small volume polyalkylimide can give a good result, it will reduce the risk of complications.

METHODS

Ethical clearance of this study was established by the Ethical Committee of the Faculty of Medicine, Udayana University, Denpasar-Bali. We used the post-test only control group design. The healthy male rats (Rattus norvegicus) strain Wistar, age six months, bodyweight 200-250 g, were divided by simple random sampling into two groups,

namely, control dan treatment groups which are 15 rats in each group. The rats were acclimatized for one week. The treatment group was given once subcutaneous polyalkylimide (Bio-AlcamidTM, Polymekon, Italy) injection 0,018 mL (equal 1 mL in human) into the frontal region, whereas the control group was given once subcutaneous NaCl injection 0,018 mL. The treatments were given on the 1st day. The rats were fed for five weeks by standard diet ad libitum and placed in an individual cage (light/dark cycle 12 hours, temperature 28±1°C, humidity 50±5%) at Animal Unit of Biochemistry Department, Faculty of Medicine, Airlangga University, Surabaya. The rats were sacrificed after five weeks by ketamine anaesthesia injection. The skin tissue was collected with a dimension 10 mm × 10 mm × 5 mm (length × width × thickness). The skin tissues were processed for hematoxylin-eosin (HE) staining at Histology Department, Faculty of Medicine, Airlangga University. Descriptive statistics and normality test Shapiro-Wilk ($p<0.05$) by SPSS 17.0 version. Histologic examinations were performed by 40× and 100× microscope magnification by a histologist. Collagen capsule thickness was performed as mean ± SD.

RESULTS

The distribution of collagen capsule data were normal (p=0,995). The treatment group showed polyalkylimide mass in subcutaneous tissue, collagen fibers surrounding the polyalkylimide, and no inflammatory cells (Figure 1). The collagen capsule thickness was 7.45 ± 0.78 µm. The control group did not show collagen capsule formation (Table 1).

DISCUSSION

Dermal fillers are the minimally invasive treatment to improve youthful facial appearance. Dermal

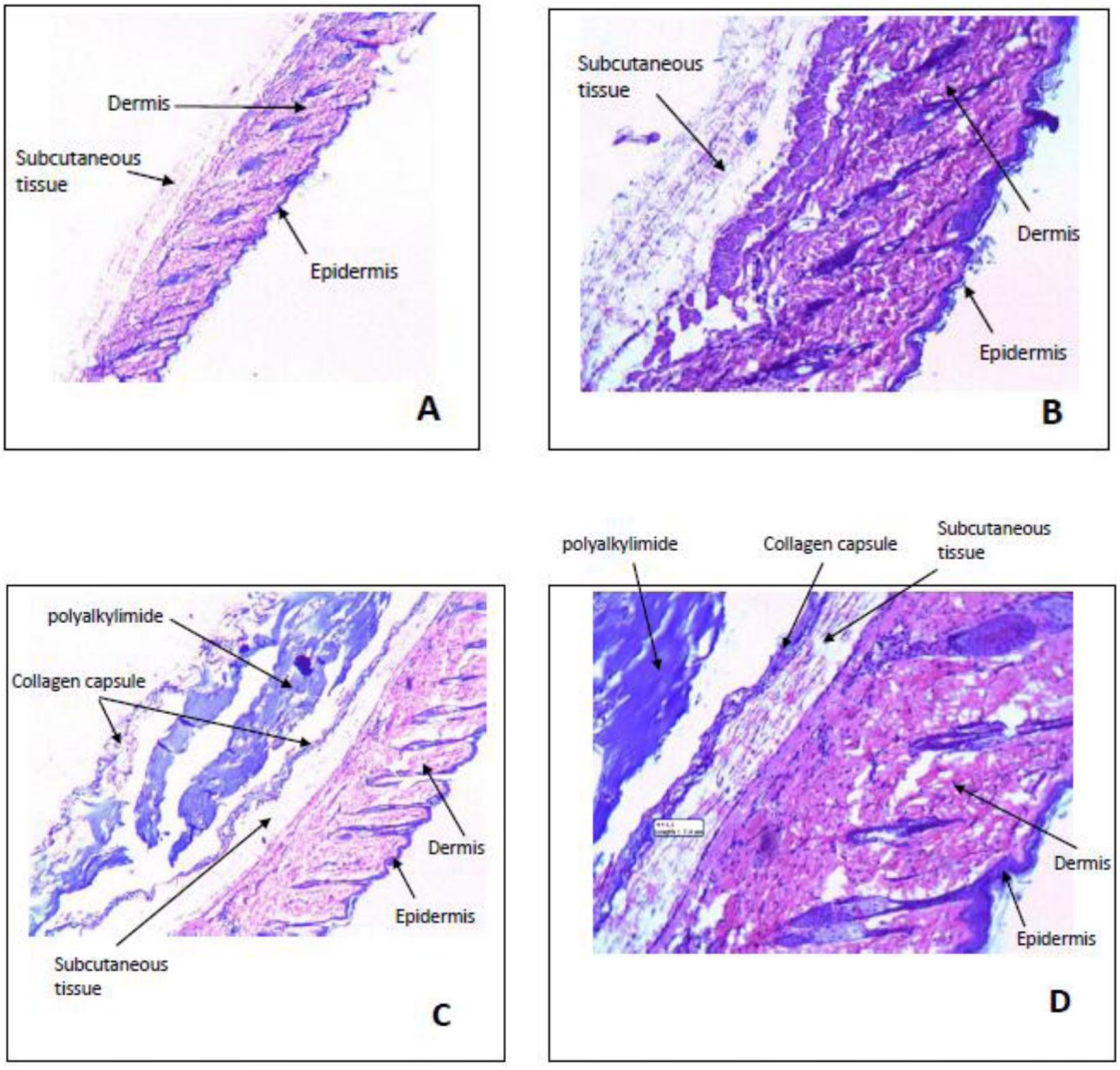

Figure 1 The histological staining of the skin. The control group, A. 40× magnification, B. 100× magnification. The treatment group, C. 40x magnification, D. 100x magnification

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Table 1 Collagen capsule numbers

Groups Collagen capsule	Control	Treatment
Negative	15 (100%)	0 (0%)
Positive	0 (0%)	15 (100%)
Total	15	15

fillers are used to improve moderate to severe skin wrinkles and folds. The additional indications are to correct the facial and the body lipoatrophy in HIV patients, acne scarring, nasal reconstruction.[7,8] Dermal fillers have the risk of complications, namely short-term and long-term. Short-term complications occur immediately up to several days. They are injection site reactions (erythema, oedema, pain, bruising, itching), infection (erythema, oedema, pain, papule, nodule, abscess), hypersensitivity (erythema, oedema, pain, non-fluctuant nodules), asymmetric face or contour irregularities or lumps, skin discolouration (redness, whiteness, hyperpigmentation), local tissue necrosis caused by vascular occlusion. Long-term complications occur in weeks to years or late-onset (delayed). They are infection (erythema, oedema, pain, nodule, abscess, systemic reaction, biofilm), foreign body granuloma, migration of implant material, immune reactions (local and general), persistent discolouration, persistent scarring, malar edema.[8,9,10]

The foreign body induced an acute inflammatory reaction at the implant site. It stimulates polymorphonuclear (PMN) cells chemotaxis and proinflammatory cytokines release, coagulation cascade and platelet activation, complement system activation. The acute inflammation resolves typically in 1 week then continue chronic inflammation. It stimulates mononuclear cells present, extracellular matrix production, and granulation tissue formation. It causes fibroblast proliferation and enhances growth factors production. The chronic inflammatory reaction resolves typically in 2 weeks. If the inflammation continues for more than three weeks, it indicates an infection.[11,12]

Several studies report complications of polyalkylimide, e.g. in prospective studies; there were implant migration, infection, oedema, bruising, pain; in retrospective studies, there were abscess, implant migration, inflammatory nodules. The complications occur a few months until 52 months.[12,13] polyalkylimide induces collagenases but has a higher risk of complications such as granuloma.[13] A case report by Nathoo et al., 2014, showed the complication of dermal fillers injection. Some patients suffered peri-ocular mass after hyaluronic acid and polyalkylimide injections.14 The study by Loutty et al., 2011, showed that most patients were satisfied with polyalkylimide gel injection. Delayed complications occur in 4 years follow-up, e.g. infections (15.6%), nodules (25%), and bleeding (3%).[15]

This study showed that small volume subcutaneous polyalkylimide injection-induced collagen capsule and no inflammatory signs after five weeks. The study by Ramires et al., 2005, reported oedema, mild hyperemia, leukocyte infiltration, granulation tissue formation without giant cells on the 7th day, the inflammatory reaction was reduced, and connective tissue was formed surrounding the implantable material on 14th day, no inflammation, necrosis, and granuloma on 30th day.[16] Present study similar to the study by Ellis and Sardesai, 2008, that showed no leukocyte infiltration. The acute inflammation occurs after implantation, then reduced and followed by connective tissue formation.[17]

Histologic examinations and immunostaining from patients with severe granulomatous foreign-body reactions after permanent fillers injection showed patients with polyalkylimide had granulomatous inflammation. However, the grading of inflammatory infiltrates does not correlate with the clinical features of inflammation.[18] Patients with an acute inflammatory response after polyalkylimide implantation showed giant cell invasion in and around the material. Patients with chronic inflammatory response showed neutrophilic infiltration in the extruded material. However, most patients showed no immune response after many years of implantation.[19] Radiologic examinations can be useful in case of polyalkylimide complications. Polyalkylimide appears hyperintense on T2 W and hypointense on T1 W sequences and reveal no-post-contrast enhancement by magnetic resonance imaging (MRI). Polyalkylimide appears as a well-defined area of fluid attenuation. This can be understood because polyalkylimide is abundance with water.[20]

Dermal filler stimulates growth factors production and enhances collagen I and III formations. Dermal filler enhances growth factors production, e.g. connective tissue growth factor (CTGF), transforming growth factor-β1 (TGF- β1), TGF-β2, TGF- β3.2,21 The previous study showed that large volume polyalkylimide implantation induced collagen capsule in 2-6 weeks with the thickness of 20 μm.3 The collagen capsule in this study was thinner than the previous study. We need further investigation of the risk of migration because of the thin collagen capsule. Dermal filler injection for an older person can still induce structural improvement of the skin due to mechanical support. Dermal filler stimulates collagen production through the TGF-β signalling pathway. It stimulates fibroblast

proliferation, up-regulation of TGF-β receptor, and growth factors production. Dermal filler is beneficial for skin correction in the elderly because collagen fragmentations increase by ageing process.[19,22]

Asian people seek beauty treatment to optimize intrinsic Asian ethnic features or correction of specific structural features. Asian patients seek dermal filler treatments to correct facial shape, nose, cheek, chin. The proportion of younger Asian patients, age 18-40 year,) increased from 44% in 2005-2009 to 48% in 2010-2014. The other reasons for aesthetic treatments seeking are to prevent or reduce ageing, follow their peer or social influence, more easily to access aesthetic products, the safety of injectable treatment over the past five years. According to patient age, treatments expectation or target can be different, e.g. 18-30 years for nasal shape, 31-40 years for tear trough and upper facial lines/nasolabial folds, 41-55 years for tear trough/malar volume loss and nasolabial folds, > 55 years for malar volume loss and jowls. In Asian people, the wrinkle slowly develops at age 40-50 years then quickly increase after 50 years. Overall, Asian face shows slower skin ageing signs than Caucasians on the third – sixth decades age.[22,23]

Younger Asian patients seek dermal filler treatment for facial restructuring, but older patients for reducing the ageing appearance. Most older patients seek treatments to restore the mid and lower facial areas due to age-related volume loss.[23] Asian face has characteristics namely weaker facial skeletal framework, wider and rounder face, higher eyebrows, fuller upper lid, the lower nasal bridge with horizontally placed flared ala, flatter malar prominence and midface, more protuberant lips, and more receded chin.[24] We need to apply a proper dose of dermal fillers, especially polyalkylimide for Asian people based on the structural and physiological Asian skin. We expect small volume polyalkylimide can improve skin augmentation for Asian people.

CONCLUSION

The small volume polyalkylimide induced a collagen capsule with no inflammatory reaction. The small volume shows promising effects and safe for skin augmentation.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the manuscript.

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AUTHOR CONTRIBUTION

The author is contributed to the content of the study from data collection, statistical analysis, results, data synthesis, and manuscript preparation.

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