Decellularized dermis-polymer complex provides a platform for soft-to-hard tissue interfaces

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ABSTRACT To develop a soft-to-hard tissue interface, we made a decellularized dermis/poly(methyl methacrylate) (PMMA) complex by soaking the decellularized dermis in methyl methacrylate (MMA) and an initiator, and then polymerizing the MMA. The decellularized tissue was chosen because of its good biocompatibility and the easiness of suturing it, and MMA because of its hard tissue compatibility and wide use in the biomedical field. The MMA filled the cavities in the dermis and polymerized within 10 min. No leaking or polymer aggregation was observed, implying that a homogenous tissue-polymer complex had formed. The cell infiltration and the integration between the tissue and the dermis occurred *in vivo*, whereas the cells could not infiltrate the tissue-polymer complex. This implies that the interface tissue should possess both complex and noncomplex parts, where the cells infiltrate the noncomplex part and stop when they encounter the complex part, integrating the soft and hard tissue or hard polymer.

Keywords: decellularization; dermis; poly(methyl methacrylate); interface tissue engineering; tissue-polymer complex

1. Introduction

Preparing a tissue interface that is biologically compatible with soft tissue and hard tissue has been a hot topic in recent years. The purpose of preparing an interface tissue is to regenerate or repair the damaged zone between different types of tissues [1]. The tendon-bone, ligament-bone, and cartilage-bone interfaces possess certain areas that have a gradually changing gradient structure. The preparation of an interface tissue is important because such a material can function as the intermediate for soft tissue integration to the dental materials, percutaneous device, stent, or any artificial materials that should be implanted into the living body. Materials such as collagen/hydroxyapatite, gelatin/hydroxyapatite, polyethylene glycol hydrogel, poly(2-hydroxyethylmethacrylate), polyacrylamide, polycaprolactone, and poly(lactic acid-co-glycolic acid) are used for the purpose of preparing an interface tissue [2-9].

Our research group has focused on using decellularized dermis as the base material in the tissue interface. This is because decellularized dermis is composed of a high percentage of collagen (97%), which is compatible with soft tissue. Decellularized soft dermis is non-inflammatory, mechanically tough, and has good cell adhesion and infiltration abilities, which made this material suitable for clinical applications [10-13].

Furthermore, it shows good integration with the host tissue, which makes it a good candidate for soft tissue compatibility. This implies that the material possesses the crucial components for the soft-to-hard tissue interface, which include a suitable microenvironment for cellular proliferation, ingrowth, and differentiation, and it meets the mechanical requirements of the insertion site. [1] For the polymer, we chose poly(methyl methacrylate) (PMMA) because of its long history of biomedical application, especially in the dental field, where it is used as a hard tissue-compatible material [14-16]. We therefore investigated ways to use decellularized tissue and PMMA to make a decellularized tissue-polymer complex to provide a platform as a tissue interface.

The most convenient method to complex decellularized tissue and polymer is to let the tissue absorb a polymer solution and then interlink the polymers. Although such a method may appear very simple, it is not the case. The problems associated with this process include the anchoring and the blending of the materials. Unless using physical force to let the polymer solution to penetrate into the native dermis, it is difficult for the polymers to be absorbed. Even if the polymer had been absorbed, lack of any sort of physical interaction between the tissue and the polymer allows the polymer to be kept inside the tissue. By contrast, we allowed the tissue to absorb a monomer solution, and

then polymerized the monomers (Scheme 1). The monomers that fill the cavities that were created when the cells were removed can be polymerized and anchored to the extracellular matrix of the native tissue. We reported the incorporation of the 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer with a cross-linker into a decellularized blood vessel [17]. We could not, however, control the amount of polymer that was absorbed, or regulate the cross-linking rate. This made it difficult to characterize the decellularized blood vessel-polymer complex.

Saturating the decellularized tissue with monomers and then polymerizing them is a much more promising method than incorporating a polymer. Such a method would provide an intermediate phase that possesses compatibility with the soft and hard tissue, or soft tissue and the polymer. That is, the monomers would fill the cavities and polymerize within them, producing a homogenous blend of synthetic material and native tissue. An advantage of using decellularized tissue is the ease of suturing it during implantation. Furthermore, it is expected to result in cell infiltration and integration with the surrounding tissue without serious inflammatory responses [18].

However, we do not know whether it is possible to prepare such a structure or how the host tissue would respond to the decellularized tissue-polymer complex. Here, we discuss the decellularization of skin tissue (dermis) and the preparation and

characterization of a decellularized dermis-polymer complex using methyl methacrylate (MMA), including that of its behavior *in vivo*.

2 Materials and methods

2.1 Materials

Fresh porcine skin was obtained from a local slaughterhouse (Tokyo Shibaura Zouki, Tokyo, Japan). The epidermal tissue and fat were trimmed, and the dermis was washed in phosphate-buffered saline (PBS) (Invitrogen, Tokyo, Japan). MMA (Wako Pure Chemicals, Japan) was purchased and distilled under reduced pressure. Benzyl peroxide (BPO) (Alfa Aesar, MA, USA) was used as the initiator and *N,N'*-dimethyl-*p*-toluidine (DMPT) (Tokyo Chemical Industry, Tokyo, Japan) was used as a co-initiator. DNase I (Roche, Switzerland) and magnesium chloride solutions (Wako Pure Chemicals, Japan) were used for washing away the cell debris.

2.2 Preparation and characterization of the decellularized dermis

Preparation of the decellularized dermis

The decellularized dermis was prepared using the high-pressure methods that have

been used to decellularize blood vessels and cornea [19-21]. In short, the dermis was placed in polyethylene bags containing PBS. After sealing, they were hydrostatically pressurized at 980 MPa at 30°C for 10 min using a cold isotactic pressurization machine (Dr. CHEF, Kobe Steel, Ltd., Hyogo, Japan) to dismantle the cells. Then, the specimens were washed by continuous gentle shaking in saline with 0.2 mg/mL DNase I and 50 mM magnesium chloride at 37°C for 10 d, treated with 80% ethanol in saline at 4°C for 3 d, treated with a citric acid buffer at 37°C for 3 d, and stored in PBS at 4°C until further use.

Characterization of the decellularized dermis

To observe the structure and cells remaining after decellularization, the specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, and cut into sections that were 4 μ m in thickness. These sections were stained with Mayer's hematoxylin and eosin (H-E) stain and elastica van Gieson (EVG) stain. The stained sections were imaged using microscopy (Coolscope, Nikon Co., Ltd., Tokyo, Japan). To collect quantitative data on the residual cells in the tissue, native and decellularized dermis samples were freeze-dried and suspended at 55°C for 12 h in 0.5 mL of a lysis buffer containing 50 μ g/mL protease K, 50 mM Tris-HCl, 1% (w/v) sodium dodecyl sulfate

(SDS), 100 mM NaCl, and 20 mM ethylenediaminetetraacetic acid disodium salt (EDTA-2Na). The DNA was extracted with phenol/chloroform and purified by ethanol precipitation. The residual DNA content was measured at 260 nm using an ultraviolet (UV)/visible-light spectrophotometer (V-560, JASCO, Tokyo, Japan).

To determine if the monomer can be absorbed by the decellularized dermis, we immersed the freeze-dried sample in MMA. Then, the sample was removed to measure the change in the weight. Measurements were taken until no further increases in the weight occurred. The same experiment was repeated with polymer/acetone mixture and water to compare the absorptivity of the decellularized dermis. The liquid content was calculated using the equation written below:

$$\text{Liquid content (\%)} = (W_t - W_d) / W_d \times 100$$

where W_t is the weight of a sample at certain time and W_d is the dried weight of that sample.

2.3 Preparation and characterization of PMMA and decellularized dermis/polymer complex

Polymerization of MMA

The polymerization of the MMA was executed by the method that was reported for

its application as bone cement [16,22]. After MMA was distilled under reduced pressure to remove inhibitors, the MMA with BPO and MMA containing DMPT were deoxygenated by argon before mixing them together in a 200-mL flask. The molar ratios of MMA, BPO, and DMPT are shown in Table 1. The molar ratio of MMA and BPO was fixed at 200:1. They were polymerized at 25°C for 10 min to produce PMMA. The temperature during the polymerization was measured by thermography (with a MobIR[®] M8 thermal camera, China) every 1 min. After the polymerization, the PMMA was obtained by dissolving the polymer in acetone and precipitating it in methanol. Gel permeation chromatography (GPC) analysis was performed at 40°C on a Shodex GPC-101 high-speed liquid chromatography system equipped with a guard column (Shodex GPC LF-G), 2 30-cm mixed columns (Shodex GPC LF-804, exclusion limit = 2×10^6), and a differential refractometer (Shodex RI-71S) to determine the molecular weight and polydispersity of the PMMA. *N,N*-dimethyl formamide (DMF) containing 10 mM LiBr was used as an eluent at a flow rate of 0.8 mL/min. The GPC system was calibrated using PMMA standards (Shodex, STANDARD M-75, $M_p = 2.89 \times 10^3 - 9.65 \times 10^5$).

Preparation of decellularized dermis/polymer complex

Decellularized dermis ($0.2\pm0.05\text{g}$) was freeze-dried under reduced pressure overnight and then put into an MMA, BPO, and DMPT mixture at the molar ratio of 200:1:1 with 1g of MMA as the standard. Then, the mixture with decellularized dermis was stirred vigorously for 10 min at 25°C. After polymerization, the specimen was taken out from the mixture and put into the methanol to remove the monomer from the matrix before rinsing with water. To measure the molecular weight and polydispersity of the PMMA in the dermis, the complex was immersed in acetone for 1 d. Then, the solution was poured into methanol for reprecipitation. The conversion ratio, molecular weight and polydispersity of the PMMA in the dermis were then determined using the same GPC analysis procedure described in the preceding paragraph.

Characterization of the decellularized dermis/polymer complex

The water content of the complex was determined by allowing samples to swell in water at room temperature for 24 h and then calculating their water content percentages by using the following equation:

$$\text{Water absorption ratio (\%)} = (W_h - W_d) / W_d \times 100$$

where W_h is the hydrated weight of a sample and W_d is the dried weight of that sample.

To investigate the homogeneous distribution of the polymer within the dermis, we

mixed Lipiodol[®] (Guerbet, Villepinte, France) as a radiocontrast agent. After polymerization, the image of the sample containing Lipiodol[®] was obtained by micro computed tomography (m-CT) imaging using a microfocus X-ray CT system (inspeXio SMX-90CT, Shimadzu, Tokyo, Japan). Computer software (VGStudioMax 2.0, Nihon Visual Science, Inc, Tokyo, Japan) was used to visualize the 2-dimensional X-ray section images of the layered scaffolds.

After a cutter was used to make a small dent on the sample surface, the samples were fractured and freeze-dried overnight. The fractured surface of the complex was sputter-coated with gold and observed using a scanning electron microscope (SEM) (S-3400N, Hitachi High-Technologies Co., Tokyo, Japan).

Compression properties were measured by a creep meter (Yamaden Co., Ltd., Tokyo, Japan). The samples were allowed to swell in water at room temperature for 24 h and then were compressed at 0.05 mm/min. Each sample was measured at least 5 times, and the measurement results were converted to stress-strain curves.

Preparation of and characterization partial decellularized dermis/polymer complex (partial complex)

A small portion of the MMA, BPO, and DMPT mixture (200:1:1, 400 μ L) was

dropped on the central part of the freeze-dried decellularized dermis (15 mm), which had been punched out (8 mm). Then, we let the mixture filtrate into the decellularized dermis and polymerize for at least 2 h. After polymerization, the specimen was put into methanol bath to remove the monomer from the matrix before rinsing with saline. The distribution of the polymer within the dermis was investigated using Lipiodol[®] as a radiocontrast agent and the microfocus X-ray CT system as described. No cross-linker was used during this process.

2.4 *In vivo* study

The animal study was performed in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publication 85-23 Rev. 1985) and the Tokyo Medical and Dental University guidelines. Wistar rats (7-week-old males; weight, 250 g) were divided into 4 experimental groups. After the animals were anesthetized with medetomidine (0.4 mL/kg, Zenyaku Kogyo, Tokyo, Japan) and sodium pentobarbital (0.3 mL/kg, Kyoritsu Seiyaku, Tokyo, Japan), they were shaved and disinfected with 70% ethanol. Then, 4 different incisions (approximately 1.5–2 cm) were made on the backs of the rats. Decellularized dermis, PMMA, complex, and partial complex (radius = 1.2 cm), sterilized with high pressure in

buffered saline (pH 7.4, 980 kPa), were implanted subcutaneously into the incision sites ($n = 3$), which were then immediately sutured. After 4 weeks, the rats were killed using an overdose of ethyl ether. The rat tissue containing the samples was excised from the rats. The samples were put into xylene to dissolve the PMMA before H-E staining. The stained sections were imaged using microscopy (Coolscope, Nikon, Tokyo, Japan). The sample preparation and staining procedures we used were the same as those used by the technicians in the Applied Medical Research Laboratory (Osaka, Japan).

3 Results

3.1 Decellularization of dermis

Dermis samples before and after decellularization are shown in Figure 1. Cells (shown as blue dots in Figure 1b) are not visible after the decellularization (Figure 1e). The decellularization process did not disturb or break the extracellular matrix (ECM) structure. The collagen fibers and elastin fibers were maintained, and no trace of debris was left behind. Hashimoto *et al.* and Negishi *et al.* have shown that the ECM structures would not be disrupted under the right circumstances [23,24]. Ice clusters that form during the pressurization could disrupt the ECM [11], but our experimental conditions

avoided their formation.

Figure 1g shows the residual DNA contents before and after the decellularization. The residual DNA content, which represents the remaining cell debris, is very low. The elimination of the cell debris depends on how much the cell membrane was disrupted and the decellularized tissue was rinsed. The high pressure only disrupts the cell structure [16], and the pressure-treated tissue should be washed well. Our high hydrostatic pressurization (HHP) method is adequate for eliminating cell debris, and it greatly reduces the residual DNA cell content.

Figure 2 shows the water, MMA, and polymer/acetone absorption behavior of the decellularized dermis. MMA can be adsorbed into the dermis, but not as much as water. The PMMA/acetone could not be absorbed by the dermis. The equilibrium was reached within 3 h for water and MMA, and there was not a significant change in the weight afterward. The change in the volume was not observed during the water, MMA or polymer/acetone absorption

3.2 Characterization of PMMA and the decellularized dermis/polymer complex

The characteristics of PMMA from bulk polymerization are listed in Table 1. Supplementary Figure 1 shows that the polymerization with a high DMPT ratio

increased the polymerization time. The polymerization heat remained below 41.5°C, implying that the denaturation of the collagen would not occur. The conversion percentage and the average molecular weight did not show significant difference among the samples, and they were between 68% and 71%. Molecular weights of specimens were between 7.2×10^4 and 7.8×10^4 .

The molecular weights and the conversion percentages of PMMA within the decellularized dermis/polymer complex are shown Table 2. The molecular weight of PMMA in the complex was 5.6×10^4 with a conversion percentage of approximately 75.8% for the decellularized dermis/polymer complex. Comparing the conversion for PMMA in the complex with that in Table 1, one sees that the conversion percentage was slightly higher but the average molecular weight was lower than those of the bulk PMMA.

Figures 3 shows macroscopic (a and c) and microscopic (b and d) images of decellularized dermis and decellularized dermis/polymer complex. No change in size or shape was evident after the complexes were made. Their surfaces were rigid, but they were not as hard as those of the bulk PMMA. The color of the complex was yellow, implying the existence of PMMA and DMPT within the dermis. The fractured surfaces in the central part of the dermis and the complex show that the ECM structure (fibrils)

within the dermis disappeared and became a monolith-like structure (Figure 3b and d). That is, pores (voids) between the fibrils disappeared, and a nonporous structure developed. We could not find pores, indicating monomer or polymer leakage, when the complex was immersed in water or any aqueous solutions over 1 week. The distribution of the polymer within the matrix was confirmed using a radiocontrast agent (Figure 3e and f). The white color implies the existence of the PMMA, and the polymer is homogenously distributed within the dermis.

The uniform distribution of the PMMA within the dermis affected the water absorption ratio (Figure 3g). When the water absorption ratios of the decellularized dermis, complex, and the PMMA block were compared, the complex behaved like PMMA. The water absorption ratio was lower than 20% for the complex, whereas it was approximately 170% for the decellularized dermis. The change in the volume for the complex was not observed indicating that the complex would not swell during the implantation period, which may cause the failure of the device.

The compression test results are shown in Figure 3h and Table 3, which show that the decellularized dermis/polymer complex is much stiffer than the native dermis and the decellularized dermis. The strain of the complex was at most 20%, while that of the native dermis was 40%. We observed the existence of an elastic phase (initial phase,

arrow), which shows low stress upon compression that is characteristic of lightly stressed native tissue [19]. The Young's modulus at this phase was almost the same for native dermis and decellularized dermis, but showed approximately 10-folds and 138-folds increase for the complex and PMMA, respectively. The Young's modulus at the collagen phase (later phase)—shown by the part of the curve where the stress suddenly increases—is almost similar among the samples. The ratio of later phase/initial phase was lowest for the PMMA, whereas the highest was shown for decellularized dermis.

The photographic and radiocontrast images of the partial complex are shown in Figure 4. The yellow part, which represents the existence of the PMMA, can be seen in the middle (Figure 4a). Figure 4b and c show that the MMA penetrated into the decellularized dermis vertically and polymerized. The major part of the PMMA exists around the hole that was made artificially.

3.3 Biological characterization

Of all the rats tested for each samples, no rats had died during the implantation period. No swelling or the hemorrhage around the implanted sites was observed. Figure 5 (a) to (c) shows the result of implanting the decellularized dermis, PMMA, complex,

and partial complex after 4 weeks. For the decellularized dermis [Figure 5(a)], the cells had infiltrated very deeply into it. The dermis integrated with the surrounding tissue, making it difficult to judge the interface between the implant and the surrounding tissue. The case of PMMA is shown in Figure 5 (b). The infiltration of the cell was not observed at all. Instead, we observed the formation of a cell line along the surface of the PMMA. The PMMA easily detached from the tissue, and the formation of the encapsulated tissue was observed. In the case of the complex, the cell surrounded the complex, and the surrounding tissue adhered weakly to the complex. We observed signs of macrophage recruitment. **The decision on the existence of macrophage was executed based on the shape of the cells formed around the implants.** The adhesion of the cells and the newly-formed tissue was observed, and cells infiltrated the shallow level of the complex.

Figure 5 (d) shows the living body response to the partial complex structure after 4 weeks. The outermost part of the partial complex, where there is no PMMA had almost the same behavior as that of the decellularized dermis as shown in Figure 5 (a). The partial complex integrated with the surrounding tissue without any significant sign of an inflammatory response on the noncomplex side, whereas macrophages formed on the complex side. The cells integrated into the matrix, but the infiltration stopped where the

PMMA existed. No sign of disaggregation between the complex part and noncomplex part was observed.

4 Discussion

4.1 Complex formation of PMMA and the decellularized dermis

As mentioned in the Introduction, our idea for the development of a tissue interface is based on making decellularized tissue-polymer complexes that are compatible with both soft tissues and hard tissues or soft tissues and synthetic materials. Because decellularized tissue shows good biocompatibility, we used it as a base material for the device. The structure of decellularized tissue is very important in our case. This is because we want to fill the cavities, which are created by cell elimination, with monomers and polymerize them to firmly anchor the polymer to the ECM. Since, the dermis is mainly consisted of collagen, the polymer would mainly anchored to the collagen fibers. Our tests have shown that using HHP to decellularize dermis maintains the ECM structure and removes virtually all of the cells (Figure 1g). The collagen fibers and elastin fibers remained undamaged, implying that the high pressure did not affect the structure (Figures 1e and 1f) [24]. Use of SDS or TritonX-100 would breakdown the

ECM structure as shown in supplementary Figures 2 (for the preparation method, see the supplementary information). We are unsure whether damage to the structure would be desirable or not for our purposes, because such a damage may allow higher monomer absorption and leakage at the same time. It should be noted that as long as the cell was eliminated, the pro-inflammatory problem would not occur. However, the question of monomer leakage remains. Therefore, we decided that it is best to keep the structure as close as possible to that of the native tissue. This is also because ECM damage, such as degradation of elastin, might cause calcification [25].

The most critical problems were whether the monomers would infiltrate the native tissue and whether the polymer would be anchored to the ECM after polymerization. MMA is highly hydrophobic and therefore likely to simply form a surface coating. We thus needed to confirm that the MMA monomers infiltrated the tissue. We expected the monomer to penetrate into the tissue by capillarity action, and we have confirmed that it does so by dropping small amounts of MMA on decellularized dermis (data not shown). Monomer infiltration of hard tissue was reported by Nakabayashi *et al.* [14], who investigated tooth substrates and found that *in situ* polymerization is possible and results in good adhesion. In our study, the MMA drop infiltrated the dermis, implying that the cavities created by the cell elimination (Figures 1e and 3d) can be filled with monomers

and be polymerized homogeneously *in situ*. It should be noted that the loading amount of MMA could not be controlled at this point, because the MMA fill up the nearby cavity of dermis by capillary absorption. Since the organic solvents which can dissolve MMA cannot penetrate into the dermis, it is very difficult to produce a dermis-PMMA complex with lower amount of PMMA. This means that only heterogeneous distribution of PMMA can be obtained. On the other hand, this also indicates that restriction the MMA infiltration into dermis can be achieved, resulting in the producing of partial complex with PMMA within the dermis as shown in Figure 4.

Polymerization inside the decellularized tissue was shown to be no different from bulk polymerization (Table 1 and Table 2). That is, the conversion rate and the molecular weight of the PMMA in the complex were almost the same as those of the bulk PMMA. This implies that the polymerization would occur within the tissue without any difficulties. As shown in Figure 3d, a monolithic structure can be seen after the polymerization, indicating that the tissue-polymer complex had formed. The filling of the cavities inside the tissue did not change the whole tissue into a hard PMMA. As shown in Figure 1, the dermis exists and the ECM structure is not disrupted or cleaved by the pressurization or washing. This enables the dermis to hold the monomers and initiator within the tissue. The monomers inside the void would be polymerized within

10 min, creating a rigid region within the matrix, indicating that the polymer and collagen fibers coexist within the dermis.

The stable existence of the PMMA in the dermis can be also speculated by the compression test. **The native tissue shows the viscoelastic property upon compression.**

The initial phase usually shows low stress, but high strain percentage for the native tissue [26]. During the initial phase, the tissue offers little resistance against the compression, but the resistance increases as the load is entirely borne by collagen fibers within the native tissue. In the later phase, the slope of the stress-strain curve for is steep and almost constant, reflecting the material properties of the collagen fibers [27-29]. As shown in the compression test **result** (Figure 3h and Table 3), a small but clear elastic phase at the early stage of strain was shown for the complex but not for bulk PMMA.

Young's modulus at the later phase was almost the same for all samples. It should be noted that the similar Young's modulus at the later phase between the decellularized dermis and the PMMA makes it difficult to divide the affect the PMMA within the dermis. At the initial phase, the Young's modulus showed approximately 10 times increase for the complex compared to that to the decellularized dermis or native tissue **due to the existence of PMMA (Table 3). However, its Young's modulus was approximately 11 times lower than that of bulk PMMA. This implies that the Young's**

modulus at the initial phase is affected by both the **ECM of the** dermis and the PMMA of the complex. Furthermore, the rise of intermediate phase relatively at lower strain **for the complex** also indicates the **existence** of the PMMA within the complex.

4.2 Living body response to the implants

Unlike decellularized dermis, the complex induced the granulation of the surrounding tissue, which involves the recruitment of the macrophage around the complex (Figure 5). The cell infiltration and the lack of a pro-inflammatory response to the decellularized tissue is the typical host response where slow and gradual resorption and remodeling occur [30]. Furthermore, the cells surrounding the complex weakly adhere to the matrix and infiltrate at the outermost part. The appearance of the macrophage indicates the degradation of the matrix, which is the typical wound-healing process [31,32]. That is, phagocytosis, which was observed for neither the decellularized dermis nor the PMMA, occurred only in response to the complex. This is thought to result because the monolithic structure of the complex, where the pores that were created during the decellularization process, was filled with the PMMA as shown in Figure 3b and d. It should be noted that PMMA remained bioinert when it was used as bone cement [33].

The increase in the macrophage recruitment and the weak cell-surface interaction can be also observed with decellularized dermis that was chemically cross-linked. Oresnstein *et al.* have reported that the cross-linked decellularized dermis induced a pro-inflammatory response because of the prolonged degradation [34]. The delayed degradation by the existence of the polymer triggered the inflammatory response, which leads to the recruitment of the macrophage. That is, the change in the structural factor altered the host response, which leads to the pro-inflammatory response.

However, we could not find solid evidence of total degradation of the matrix. We only found that the cells may adhere to the surface of the complex and proliferate, which eventually induced phagocytosis. As the result, there was partial degradation of the matrix's surface. As mentioned before, the anchoring of the PMMA to the ECM made it difficult for the cells or any soluble factors within the living body to penetrate (Figure 3g and Supplementary Figure 3). With this, there was no sign of the formation of a fibrous capsule, which may lead to the device failure [32,35]. This is because the collagen fibrils, which promote the cell-matrix interaction, coexist with PMMA and suppress the interaction. The formation of the fibrous capsule leads to the loosening of the soft or hard tissue because the matrix is completely isolated from the structure. Therefore, we think that the weak adhesion of the cells on the complex triggered the

macrophage recruitment but did not lead to the degradation of the complex or any cell infiltration into the matrix.

However, the complex should be used where there is space to root to the host tissue. This is because the device needs to be stabilized in the implant site, and endure the constant stress that is caused by continuous body movement. Therefore, the partial complex, which can be stably fixed to the soft tissue and the hard tissue with a gradient structure, was prepared. We prepared a partial complex with PMMA locally concentrated around the hole (Figure 4). The concentration of the PMMA at the central part of the dermis was high, and the spreading of the MMA occurred vertically. Therefore, the distribution of the PMMA was almost the same from top to bottom, which makes it possible for the hard tissue to grow through the hole and be fixed by the interaction with PMMA.

As shown in Figure 5 (d), the tissue surrounding the decellularized dermis integrated and did not have an obvious interface, whereas the complex part showed weak cell adhesion to the matrix. Much clear images can be seen in Supplementary Figure 3 where much magnified images of implant-tissue interface are shown. The cell infiltration from the dermis part stopped at a certain depth. This matches the data that are shown in Figure 4 c, where the PMMA exists at the central part (arrow). The cells

penetrated until they encountered the PMMA, which is represented in white color. The dermis part gradually becomes part of the living body through remodeling. That is, the infiltration of the cells stops at the intermediate phase, which does not have an interface between the polymer and the tissue as mentioned in the Introduction. This can be used in the way to cement the catheter or the hard tissue in the middle of the partial complex as shown in the scheme 2. The material which is placed in the middle of the complex would produce a firm interface, disallowing the cells to infiltrate between the material and the complex. The integration between the soft and hard tissue or soft tissue and polymer occur with slow resorption of the dermis part by the living body which results in strong tissue-complex attachment would remain stable for long time. Some speculations can be made about the soft tissue-polymer interface, where the complex part may be cemented to the hard tissue or to the hard polymer, preventing cell infiltration between the complex and the hard tissue or the hard polymer which may induce the separation from the native tissue. Therefore, the dermis is stably fixed to the host tissue (soft tissue), thus allowing the device to be firmly rooted to the host tissue.

5. Conclusion

We found that it is possible to use bulk polymerization to make a complex of decellularized tissue and a synthetic polymer. The monomers filling the cavities where the cells were removed became a polymer after 10 min of the polymerization process. We showed that the polymer would be anchored to the ECM fibers and that it exists stably in the tissue. To our knowledge, this is the first study on the integration of a hydrophobic polymer with a soft tissue, and the living cells' response to it. Furthermore, we managed to prepare a partial complex which would be compatible to both soft tissue and polymer/hard tissue at the same time, functioning as an interface between materials with two very distinct physical or biological properties. We believe that this complex possesses high potential for the new type of percutaneous device.

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Scheme 1. Schematic images of the dermis structure, decellularized dermis structure and dermis/polymer complex structure.

Figure 1. Photographs (A,D) hematoxylin and eosin stained sections (B,E), and elastica van Gieson stained sections (C,F) of native dermis and decellularized dermis after thorough washing, and the residual DNA contents of native dermis and decellularized dermis (G).

Figure 2. Absorption of water, methyl methacrylate (MMA) and poly(methyl methacrylate) (PMMA)/acetone by the decellularized dermis after 120 min (A) and after 24 h (B).

Figure 3. Photographic images (A,C), scanning electron microscopy (SEM) images (B, D), and computed tomography images taken from above (E) and the side (F) of decellularized dermis. (A, B) represents the decellularized dermis and (B, D) represents decellularized dermis/PMMA complex (C, D). Water absorption ratio of decellularized dermis/PMMA complexes after 60 min (G). Stress-strain curve of the native dermis, decellularized dermis, decellularized dermis/PMMA complex, and bulk PMMA (H). The line and the arrow indicates the elastic phase upon compression.

Figure 4. Photographic images (A), and computed tomography images taken from above (B) and the side (C) of decellularized dermis and partial complex. The arrow indicates the existence of the PMMA within the dermis.

Figure 5. H-E stained histological images of the decellularized dermis (A), PMMA (B), complex (C) and partial complex after 4 weeks of implantation. The photographic images of the subcutaneously implanted dermis, PMMA and complex are shown on the left side of the histological images.

Scheme 2. The partial complex with material placed in the middle. The cell penetration stops at the complex site and integrates with the soft tissue. The complex part integrates with the hard tissue or the implanted polymer, cementing 2 types of tissue together.

Table 1. The molar ratio of MMA and DMPT, and the characteristics of PMMA from bulk polymerization

	molar rate (BPO:DMPT)	$T_{\max}$ (°C) ^[a]	$t_{\max}$ (min) ^[b]	Conversion (%) ^[c]	M_w (g mol ⁻¹) ^[c]	M_n (g mol ⁻¹) ^[c]	M_w/M_n
PMMA1	1:1	41.1±1.3	6.0	70.2±1.8	2.5×10^5	7.6×10^4	3.26
PMMA2	1:2	41.2±0.8	5.0	67.9±5.9	2.4×10^5	7.8×10^4	3.12
PMMA3	1:3	41.3±0.9	4.0	71.0±5.8	2.5×10^5	7.2×10^4	3.47
PMMA4	1:4	41.5±1.8	2.0	72.7±5.4	2.6×10^5	7.5×10^4	3.56

^[a] The highest temperature observed with thermography during the polymerization.

^[b] The time required to reach the Tmax.

^[c] Measured with GPC.

Table 2. The molecular weights and the conversion percentages of PMMA within the decellularized dermis/polymer complex

Conversion (%)	M_w (gmol ⁻¹)	M_n (gmol ⁻¹)	M_w/M_n
75.8±1.5	1.8×10^5	5.6×10^4	3.22

Table 3 Young’s modulus (KPa) of respective samples.

n=3

	Decellularized dermis	Native dermis	Complex	PMMA
Initial phase	4.1	5.6	50.6	568.5
Later phase	1159.4	1116.4	1186.4	1165.9
Later phase/initial phase	283	199	23	2