Comparison of Cellularity, Cytotoxicity, and Collagenase Resistance of DermACELL® and a Marketed Acellular Dermal Matrix Product - AlloMax®

Introduction

Acellular dermal matrices (ADMs) represent a group of allograft or xenograft products that have been decellularized and used in different clinical applications including soft tissue replacement in the breast, abdomen, and the lower extremities. DermACELL® (LifeNet Health) is an acellular human dermis product processed with a patented Matracell® technology. The resultant acellular dermis is preserved at ambient temperature to maintain the acellular dermal tissue essential characteristics and being ready to apply at clinical settings with or without any rinsing. In addition, the DermACELL product is sterilized by gamma irradiation at low temperature to ensure the sterility without compromising the biomechanical properties, biocompatibility, and functionality. AlloMax® (Bard) is a gamma irradiated sterile allograft made from human dermis that has been Tutoplast – processed and dehydrated. AlloMax can also be stored at room temperature but needs to be rehydrated prior to use.

The cell removal processes varies from company to company, which yields different ADM products with various cellularity and matrix structure. This study is to quantify and locate residual DNA in DermACELL and AlloMax, compare the cytotoxicity and collagenase susceptibility of these two products.

Research Design and Methods

1. DNA quantification

Three different clinical lots of DermACELL with research authorization and three clinical lots of AlloMax were used for this study. Grafts were prepared according to each product instruction for use (IFU). Three representative DermACELL samples from each lot of graft were lyophilized and weighed. Three representative samples were cut from each lot of AlloMax and weighed. The lyophilized tissue samples were digested with proteinase K and DNA was quantified using a PicoGreen® DNA assay kit (Invitrogen P11496) following LifeNet Health standard operation protocol. The results were expressed as nanogram of dsDNA per milligram ADM dry weight. The residual DNA data was averaged for each product group.

2. Histology

A minimum of three representative samples from each lot were used for histological analysis. Normal human skin samples from one donor were used as a control. Briefly, the samples were fixed in 10% neutral buffered formalin followed by dehydration/cleaning/paraffin infiltration. Each sample was bisected and embedded with both cross section faces facing down. The tissue was sectioned at 5 micron for hematoxylin and eosin (H&E) staining or DNA staining using a Feulgen staining kit (American MasterTech). The images of stained tissue were scanned and recorded for visual comparison among groups.

3. Cytotoxicity Assay

One representative tissue sample from each lot (one DermACELL lot and three AlloMax lots) were used for cytotoxicity assessment using L929 cells (ATCC Cat. No. CCL-1). The AlloMax tissue were weighed dry and then rehydrated in isotonic saline for 15 minutes statically. Following rehydration the tissues were weighed again to obtain the wet weight and used for extraction media volume calculation. The extraction was performed by immersing each piece of tissue in Eagle's Minimum Essential Medium (EMEM) containing 1% Penicillin/Streptomycin (P/S) for 24 hours at a 1:5 (w/v) ratio with gentle agitation (28 RPM) at 37˚C according to ISO 10993-12. A media control group without tissue was also incubated alongside the tissue extraction. Following extraction, fetal bovine serum (FBS) was added to the extracted media to reach 2% FBS for each group and then the extracted media was transferred to 12 well plates containing L929 cells, seeded at 2000 cells/cm^2 the day before.
On days 1, 3, and 6, the cells were observed under microscope and photos were taken for record. Then cells were detached from the plate and the cell pellets were used for DNA quantification using the PicoGreen DNA assay kit as described in the DNA Quantification section. The quantity of chromosomal DNA was used as an indication of the L929 cell number. Data are expressed as a mean +/- standard error of the mean.

4. Collagenase Susceptibility Assay

Three clinical lots of Allomax were cut into 9 squares of about 50mg each (dry) per lot and weighed. One lot of DermACELL was rinsed three times in ultrapure water to remove preservation agent for 15 minutes each time with gentle rocking. The rinsed tissue was cut to 9 pieces and weighed to about 250mg each piece in wet weight, which is about 50mg each piece in dry weight according to previous dry weight and wet weight ratio calculation.

Solutions of collagenase type 1A (Sigma-Aldrich C9891-100MG) were made in tris-buffered saline (TBS) pH 7.6 containing 2mM CaCl2. Collagenase solution (5mL) at 100 CDU/mL, 50 CDU/mL or 0 CDU/mL were added to each lot of tissue in triplicate. Tubes containing tissue and collagenase solution were placed on a rocker in a 37˚C incubator for approximately 16 hours. Following digestion the tubes were centrifuged at 2390 rcf for 15 minutes to collect any undigested tissue. Undigested tissue was removed from the digestion tubes, dabbed briefly on paper towel to remove surface liquid and weighed. Photographs were taken for each piece of tissue prior to and after collagenase digestion. Analysis of variance (ANOVA) and post hoc analysis was performed to assess any statistical significant difference among groups.

Results

1. DNA quantification

The average DNA quantity from 3 clinical lots of AlloMax and DermACELL were 134.87 ng and 8.23 ng per mg of dry tissue, respectively (Table 1). AlloMax has more than 15 times of residual DNA than DermACELL.

Table 1: Quantification of the residual DNA in two ADM products (Data from 3 different clinical lots of each product)

	AlloMax	DermACELL
ng DNA/mg dry tissue	134.87±14	8.23±2.04

2. Histology and DNA staining

Representative images of H&E stained and Feulgen stained samples for the two different groups (DermACELL and AlloMax) are shown in the figures 1-3. Nucleic acid stained blue in H&E staining and magenta in Feulgen staining were found in areas closest to basement membrane or hair follicles in AlloMax samples. No nucleic acid staining was found in DermACELL samples for all three lots tested. Cells with nuclei were found in hair follicles and gland areas in all 3 lots of AlloMax samples (Figure 2).

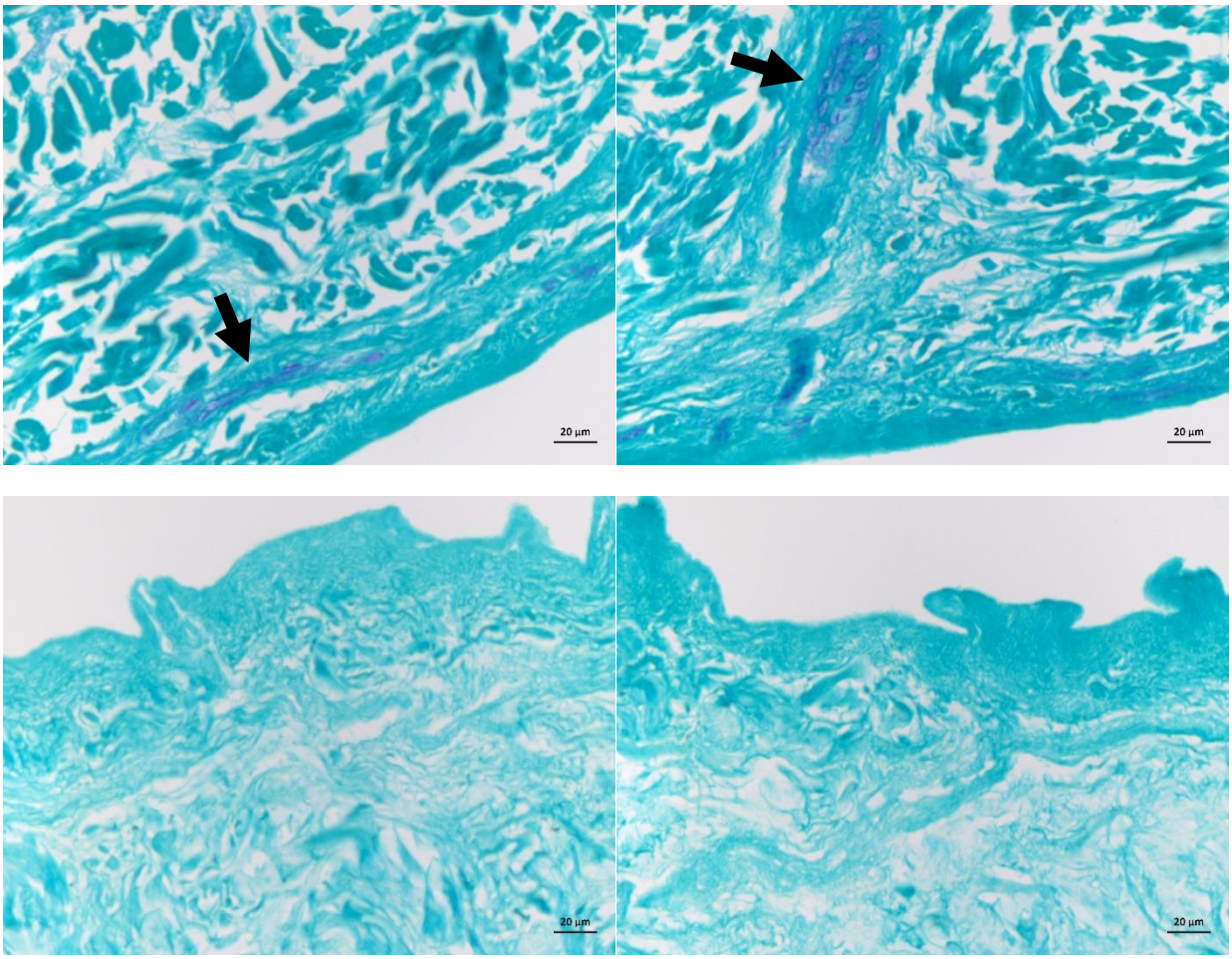

Figure1: Representative photo of AlloMax (top row) and DermACELL (bottom row) with Feulgen staining. The nucleic acid material (arrow) was stained magenta and the collagen matrix was stained green. All photos showed scale bars of 20µm.

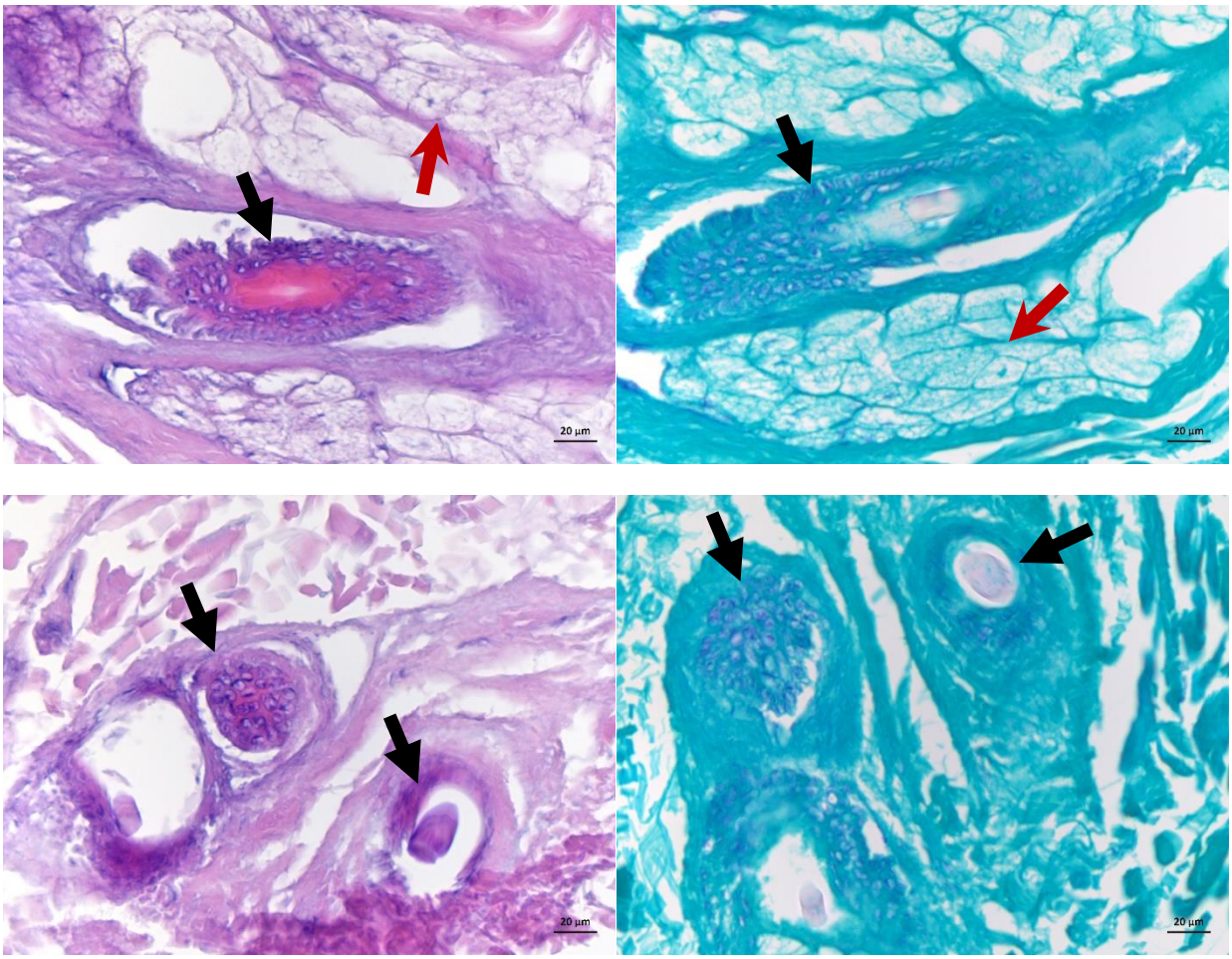

Figure 2: Representative photos of hair follicle (black arrow) and gland (red arrow) in AlloMax. The nucleic acid material was stained blue color in H&E (left) and magenta in Feulgen staining (right). All photos showed scale bars of 20µm.

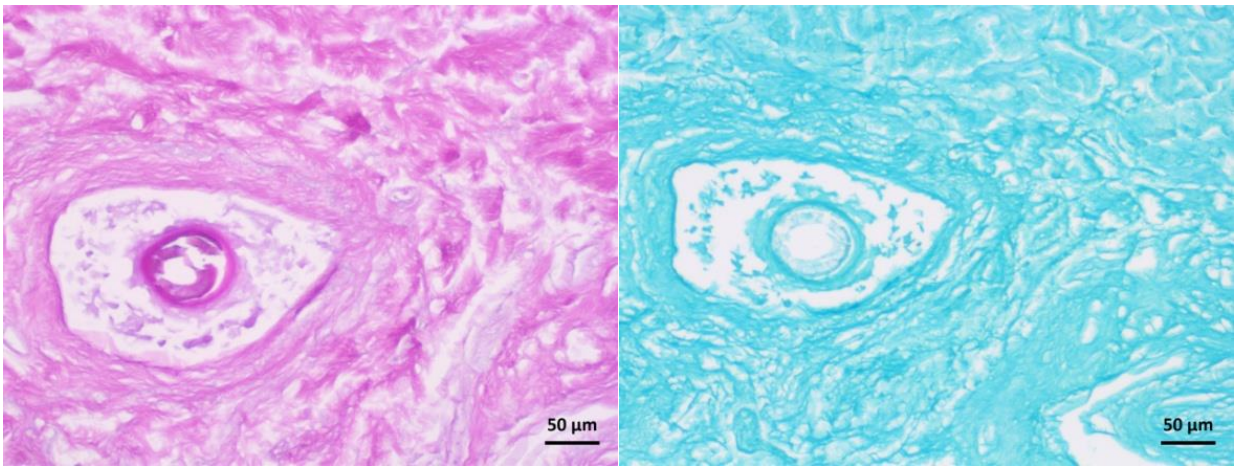

Figure 3: Representative photos of hair follicle in DermACELL with H&E staining (left) and Feulgen staining (right). No nucleic acid staining was found in either staining method. All photos showed scale bars of 50µm.

3. Cytotoxicity

All groups showed normal L929 cell morphology at days 1, 3, and 6 (Figure 4) and no significant cell death was found in any group. There was, however, some debris floating in the wells containing AlloMax tissue extraction, which was not present in the media only or DermACELL groups. No significant difference on cell growth among all tested DermACELL and AlloMax lots (Figure 5) on day 1 through day 6.

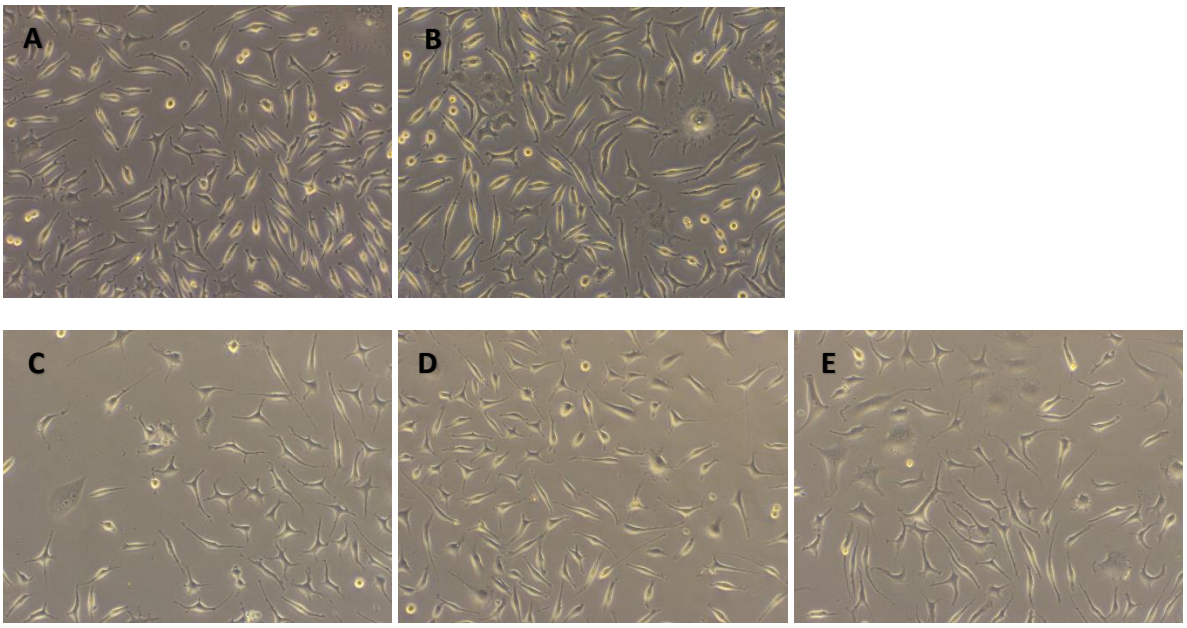

Figure 4: L929 Cell Morphology after 6 days in culture. A) Media only; B) DermACELL; C) AlloMax Lot 1; D) AlloMax Lot 2; E) AlloMax Lot 3.

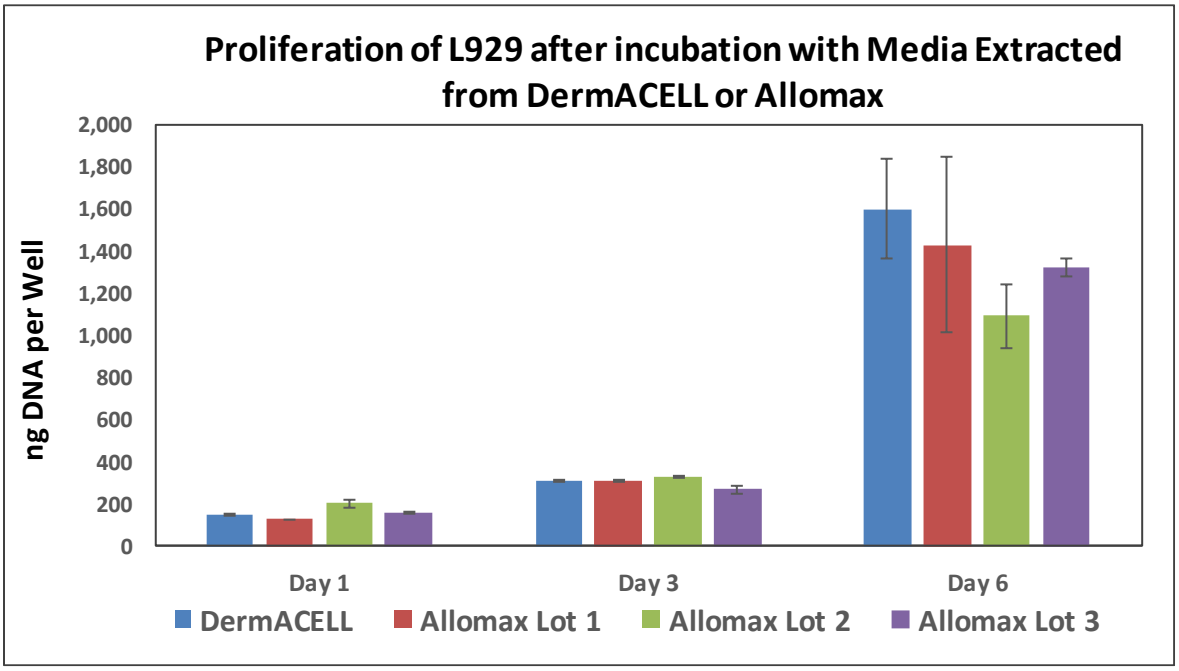

Figure 5: Quantification of DNA in L929 cells treated with DermACELL or AlloMax extraction for 1, 3, and 6 days.

4. Collagenase susceptibility

After overnight collagenase treatment, the samples in AlloMax group were almost completely digested in both 50 CDU/mL and 100 CDU/mL groups (Figure 6). Percent dry tissue mass remaining was calculated for all groups using the formula of [(final dry weight) / (initial dry weight) x 100%]. DermACELL samples were significantly more resistant to degradation by collagenase type 1A than all Allomax samples at both 100 CDU/mL and 50 CDU/mL ($p<0.01$, Figure 7).

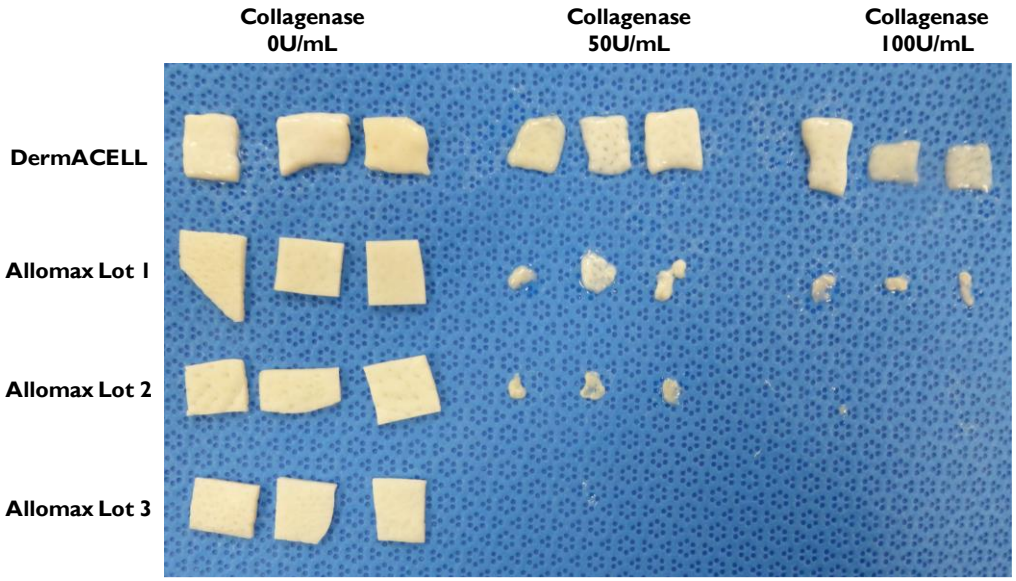

Figure 6: Tissue remaining after collagenase digestion at 37˚C overnight.

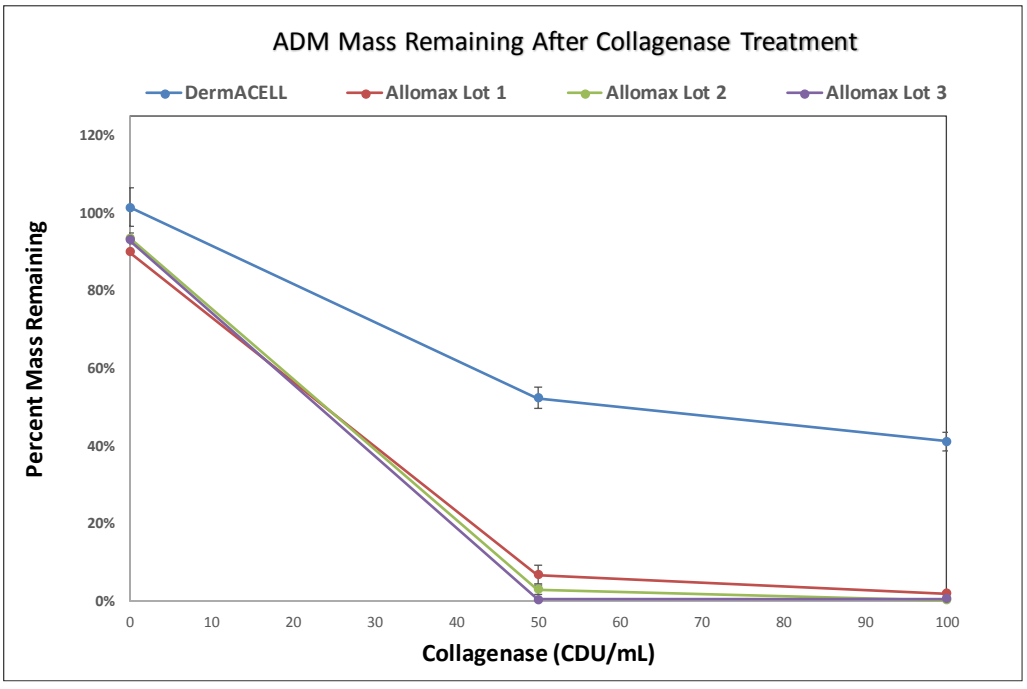

Figure 7: Percentage of tissue remaining after collagenase digestion at 37˚C overnight.

Discussion and Conclusion

AlloMax had 15 times higher residual DNA level than DermACELL, and this residual DNA was mostly located at the areas that had the highest cell density prior to the decellularization, where the hair follicles

and glands were. AlloMax also showed significant less resistance to collagenase than DermACELL at both concentrations (p<0.01), which is an indication that AlloMax had more modification from native dermal tissue structure and the potential of being absorbed much faster than DermACELL. No cytotoxicity was detected in the extraction media from both products.

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