Comparison of mechanical properties of rabbit and human skin decellular scaffolds for alternative uses in tissue engineering

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Research Article

Keywords: mechanical properties, tissue engineering, human skin, derm, rabbit.

Posted Date: July 13th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1767335/v1

Abstract

Objectives: In skin tissue engineering, there is a need for suitable tissue replacements that can be used as scaffolding. The higher similarity of the structural properties and biocompatibility of these scaffolds, the more desirable the alternative will be. Due to this, and considering that this scaffold should be used decellularized if needed, this study was performed on decellularized tissues. this study aimed to study the similarity of mechanical properties of rabbit decellularized derma with human decellularized derm.

Methods: Sampling was done in this study as available. A total of 52 rabbit specimens were obtained, which were divided into two groups: decellularized and control. Twelve human specimens were obtained, which were decellularized and controlled. The stress strain of the samples was measured by Zwick / Roell Z010 device. Then, based on the obtained values, Young's modulus was calculated and the mean values in the groups were compared by t-test. Graphpad prism Version 9.0.0 software was used for comparison. A significance level of 0.05 was considered.

Results: The results showed that there was no significant difference between the mean Young's modulus of rabbit skin samples before and after decellularization. The results also showed that there was no significant difference between the mean Young's modulus of human skin samples before and after decellularization. Finally, the results showed that there is no significant difference between the mean Young's modulus of decellularized rabbit skin and decellularized human skin.

Conclusion: According to the results of this study, detoxified rabbit skin can be a suitable alternative to human skin in tissue engineering in terms of mechanical properties.

Introduction

Today, one of the most promising fields in medicine is tissue engineering, which replaces damaged tissues. In recent years, this field has created many hopes in various subjects and researchers by relying on the use and combination of different tools from different sciences such as materials science, cellular, and molecular science, nanotechnology, etc. have made good progress.

In many clinical situations, the need for skin grafting has been raised. This transplant can be done as an autograft, allograft, or xenograft. if it is done as an allograft and not an autograft, Complications such as immunological responses, graft rejection, and multiple complications will be appear, and in the case of xenografts, there will be acute complications. Because of autografts and even allografts, skin grafting is not always possible; Therefore, in recent years, decellularization of non-autograft tissues has become very important for the following reasons: 1.Removal of immunogenic cellular factors; 2. Preservation of matrix proteins due to their protected genes; 3. Have less ability to stimulate the immune system and also have the ability to lead cell differentiation.

High in differentiating stem cells from target cells can be used in textile engineering as an excellent natural scaffold. Due to these cases, the high success rate of these scaffolds in tissue transplantation can be used with less stimulation of the immune system (1). Both cellular and decellular products can be used to manage a variety of chronic wounds, including venous and diabetic wounds, high blood pressure, and other conditions such as burns and surgical wounds (2). At present, many studies have been done on the decellularization of various tissues, including skin dermis, liver, bladder, and small intestine (3).

in the case of xenografts, it must provide the mechanical properties of the target tissue. The acellular dermal matrix, or ADM, can be considered a new membrane for correcting mucosal defects. In a study of patients with hypo-pharyngeal cancer whose bovine ADM was repaired, 11- to 36-month follow-up showed that patients started eating after 2 to 5 weeks. After 36 months, no disease recurrence was observed, and the least possible side effects occurred (4). In another study, the efficacy and safety of ADM in patients with diabetic foot ulcers were evaluated; Diabetic foot ulcer is a chronic and resistant disease; The results showed that the wound healed completely, and the mean healing time was significantly shorter compared to standard care while the side effects were not significantly different (5). Another cohort study using human ADM to treat type 2 diabetic ulcers showed complete recovery and no complications after 17 weeks of follow-up. This study was performed on three people (6). In addition, a study by Chen et al. Found that severe burns could be treated with a dermal decellularized matrix, leading to the production of the commercial DermACELL product (7). has taken. Some of these studies have even gone as far as pre-clinical trials and have been so successful that they have led to the production of the commercial OASIS product, which is derived from the small intestine of pigs (8). A 2011 study by Zhang et al. The decellularized small intestine of pigs found that the small intestine had high angiogenesis, water exchange potential, and good mechanical properties, so it had great restorative power in repairing the abdominal wall (9). Research has shown that growth factors such as basal fibroblast growth factor (FGF-2) and TGF-b, present in this area, affect vital tissue activity. Development and differentiation provide an opportunity to induce mechanisms involved in tissue and wound regeneration (10). Climov et al. Also reviewed seventy preliminary studies in an extensive study. They suggested that ADM is an advantageous model, but its optimal use requires more clinical studies in the future (11). On the other hand, despite the high value of the global rabbit market and its commercial and industrial uses, the use of rabbit skin as a medical use seems to be a suitable solution if it has the minimum alternative conditions. Therefore, in this study, the comparative effects of stretching (one of the important conditions in replacement and use as biological dressing) of decellular rabbit skin tissue in comparison with human decellular skin were investigated.

Methods

Sample preparation

Dutch Belted rabbits weighing about 2.2 to 2.5 kg were used to sample the rabbit's skin. No more than two hours had elapsed since the rabbits died. Available sampling was performed. Samples were extracted from the back and sides of three rabbits. After sampling, they were cut into smaller pieces 5×5, and some of them entered the decellularization process. Human skin samples were obtained from the surplus skin in forensic medicine with the permission of the University Ethics Committee. The deceased were aged 25 to 80 years, and due to the limited size of the sample and its number, only six human samples were prepared. Some of them were decellularized after receiving the sample, and some were retained as controls.

Decellularization and tensile testing

In this process, according to the Tissue Engineering Laboratory of Tabriz University of Medical Sciences protocol, first, the epidermis was removed, and the parts were inserted in the form of dermis only in the continuation of the process. Finally, decellularized dermis was placed in PBS solution, and after 24 hours, stress-strain was tested by the machine. Data were obtained from 26 samples of rabbit decellularized dermis and 26 samples of control dermis cut with a length of 1×5 cm. Also, six samples of human dermis were decellularized, and six samples of control human dermis with 1×5 cm long dimensions were cut and tested for final tensile strength. For this purpose, the two edges of each sample were fastened to two fixing clamps of the Zwick / Roell Z010 device) Furthermore, it stretched at a speed of 5 mm per minute in the wet state.

Investigation of Decellularization

The research samples were studied by descriptive histology and cell removal by H&E staining histology and light microscopy. For histological examination, some of the samples were randomly removed after decellularization and placed in fixation solution for 48 hours. Sample preparation protocol for slide preparation, including dehydration, was performed using ascending alcohol concentration (50%, 70%, 90%, 96%, 100%) and 5 minutes each, followed by infiltration, embedding, and sectioning steps. Finally, H&E staining was performed to examine the size of the remaining cell nuclei.

Statistical Analysis

Statistical analysis was performed to evaluate the difference between the mean tension of the control group and decellular paired-sample t-test, and to examine the difference between the mean tension in rabbit dermis and human dermis independent sample unpaired t-test was used. Analyzes were performed using Graphpad Prism software version 9. The 95% confidence interval was considered.

Results

Histology

H&E staining analysis using decellularization protocol showed that the method used was able to remove a significant percentage of skin cells in both human and rabbit samples. Figure 2 shows untreated and

H&E stained scaffolding. According to the pictures, by maintaining the scaffold, the cells effective in transplant rejection were effectively removed Fig2.

The effect of decellularization on mechanical properties

Yang modulus was calculated for both control and decellularized groups in rabbits and humans. and by comparing the means of the two groups, the results showed that decellularized human dermis group and its control, despite the changes, these changes were not significant (p = 0.063)Fig3-a. In addition there was no significant difference between the two groups of decellularized and control rabbit dermis (p = 0.123)Fig3-b. The results showed that decellularization had no significant effect on the elasticity of the groups.

Comparison of decellularized Scaffolds in Rabbit and Human

In order to evaluate the difference in tension in rabbit and human decellularized scaffolds, Young's modulus was calculated for each of them and their mean was compared by independent sample unpaired t-test. The results showed that despite the mean difference between the two groups, this difference was still not significant (p = 0.373) Fig3-c.

Table 1. Young modulus group differences between rabbit and human decellular groups

	Rabbit		**Human**		**P-value**
	Control	Decellular	Control	Decellular	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Young Modulus (MPa)	0.047±0.023	0.038±0.015	0.052±0.039	0.031±0.022	0.3733

Discussion

In this study, which was performed to compare the mechanical properties of rabbit and human decellular skins for alternative use in wound tissue engineering, the results showed that human and rabbit dermal scaffolds after decellularization process, despite showing a decrease in elasticity, the difference They have no significance in terms of elasticity. Also, in the study of the degree of elasticity between rabbit decellular dermis and human decellular dermis, it was observed that despite the larger yang modulus in rabbit skin scaffold, but this difference is not significant. When comparing the data with what is in the background, it was found that there is a big difference between the researchers' reports in terms of rabbit skin yang modulus and human skin yang modulus. Of course, this is to be expected when it comes to tissue and biological conditions, as the determinants of a person's genetics and age, as well as the nature of the skin and the test conditions. For example, in the present study, the Young's modulus for human control was 0.052 ± 0.039, but in Jansen & Rottier's research, which also examined the amount of traction in the laboratory, the Young's modulus was reported to be in the range of 1.07 to 2.157 MPa (12). However, this interval was consistent with the results of the present study in many studies. For example, in the study of Park et al. (13), the Young modulus was reported to be 0.006 to 0.0289 MPa; in the study by Tupin et al (14). A report by Koene et al.(15), Which reviewed reports of Young's modulus range of human skin, ranged from 0.02 to 0.3 MPa (21). Due to this range, the results obtained from the present study are in this usual range. The difference between the values obtained from this study and Jansen & Rottier's study can be related to the experimental conditions and tools used. Jansen & Rottier's research was conducted in 1958 with non-standardized samples. Also, in the study of these researchers, the amount of time elapsed after sampling was not mentioned; The time interval could have a large impact on the test results, and even the values reported were not within the range reported by Koene and many other studies.

The values obtained for rabbit skin were 0.047 ± 0.023, which is in the range of elasticity in human skin, and the results of the analysis showed that these values, despite the difference, are not significantly different. The aim of this study was to investigate the extent of rabbit skin elasticity as an alternative in tissue engineering applications; Since compatibility for transplantation is also important in tissue engineering, rabbit and human decellular scaffolds were also compared. The values obtained showed that after decellularization with the same method for both rabbit and human tissues, although the amount of elasticity in both species has decreased, but this range is still in the range that can provide the required elasticity in providing engineered tissue alternatives.

According to research reports, various factors can affect the amount of skin elasticity, including the location of the sample (16,17). In this study, to prevent the effect of this factor, all human samples were collected from almost one position. Also, the underlying tissues, including subcutaneous fat, are not seen in rabbits, but in humans, this layer is relatively thick. It was removed to examine only the dermis in both species. According to the literature, subcutaneous fat can affect the rate of skin contraction (12).

The time after death and the duration of separation of samples from the primary tissue can also affect the amount of skin elasticity. This study tried to test the samples quickly after sampling, and due to the sensitivity of this tissue to drought, Moisture of the samples was provided at the time of testing. Observance of all these cases in previous research has been done in only a few studies or at least reported.

This research also had some limitations. One of the limitations of this study was the type of sampling that was done in an accessible way. This in itself will reduce the generalizability of the results. Finally, for future research, it is suggested to study the biocompatibility effects of this animal scaffold on a cellular basis in order to provide the necessary conditions to replace this tissue product in tissue-engineered scaffolding.

Conclusion

The results of this study showed that rabbit skin, despite the amount of elasticity different from human skin, still covers the range of human skin elasticity. Also, after decellularization of both rabbit and human tissues, despite the decrease in the amount of elasticity in both tissues, the amount of elasticity was not significantly different from human tissue. Therefore, this scaffold can be used as an alternative matrix in tissue engineering studies.

Declarations

Compliance with Ethical Standards:

This study was funded by Tabriz medical University of Sciences (Grant number: IR.TBZMED.REC.1397.143)

Conflict of Interest: Authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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Figures

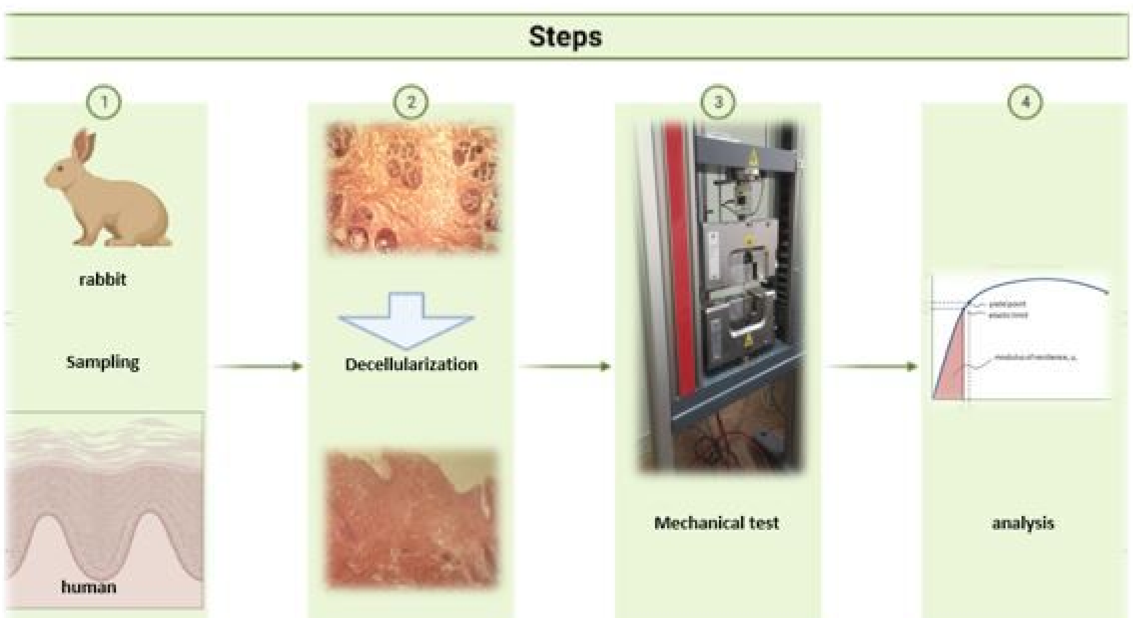

Figure 1

Project steps briefly.

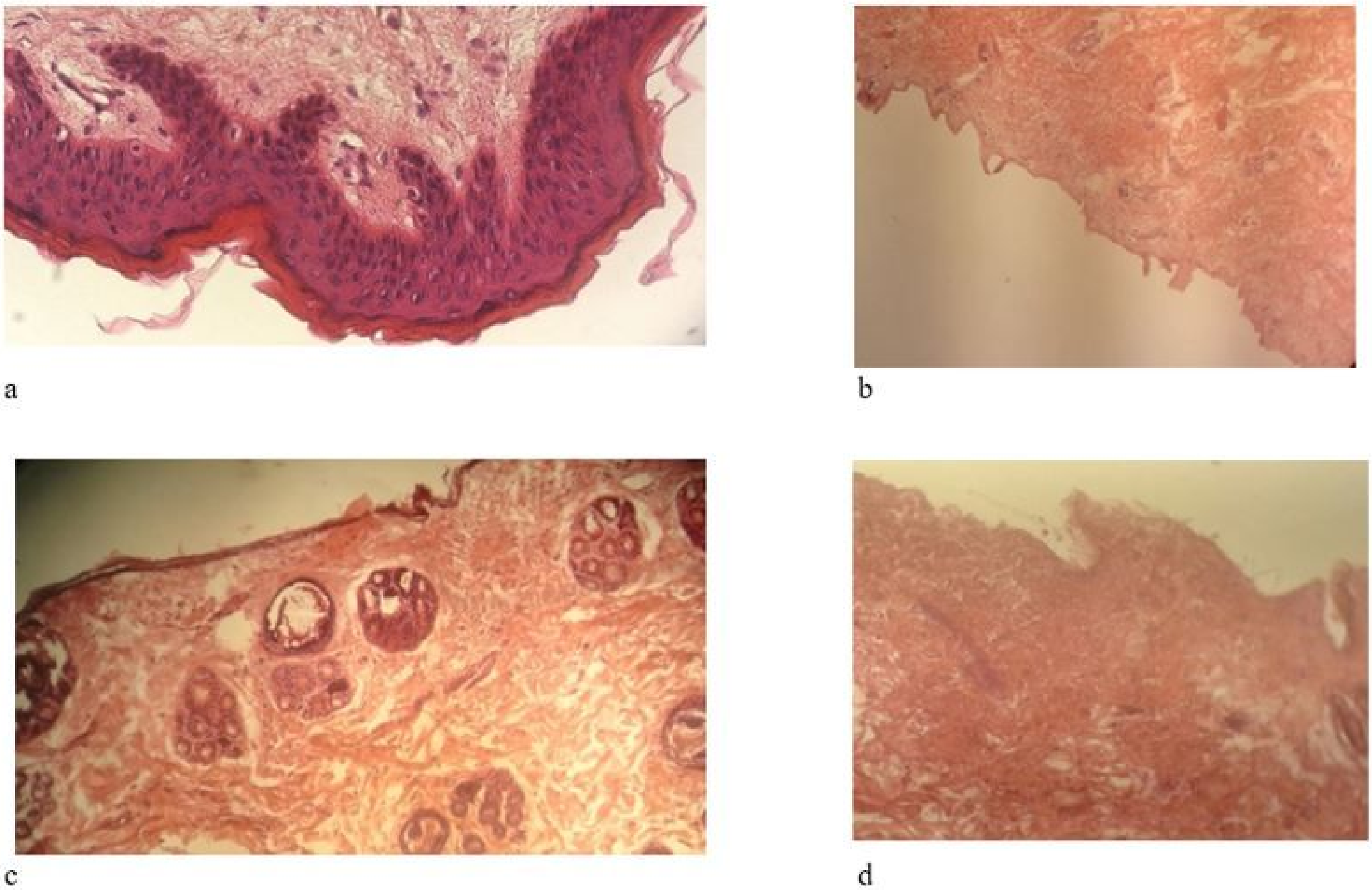

Figure 2

Optical micrograph of H&E staining of human specimens (a and b) and rabbit specimens (b and c). a) Human derm (HD) before decellularization. b) Human derm (HD) after decellularization. c) Rabbit Derm (RD) before decellularization. d) Rabbit Derm (RD) after decellularization.

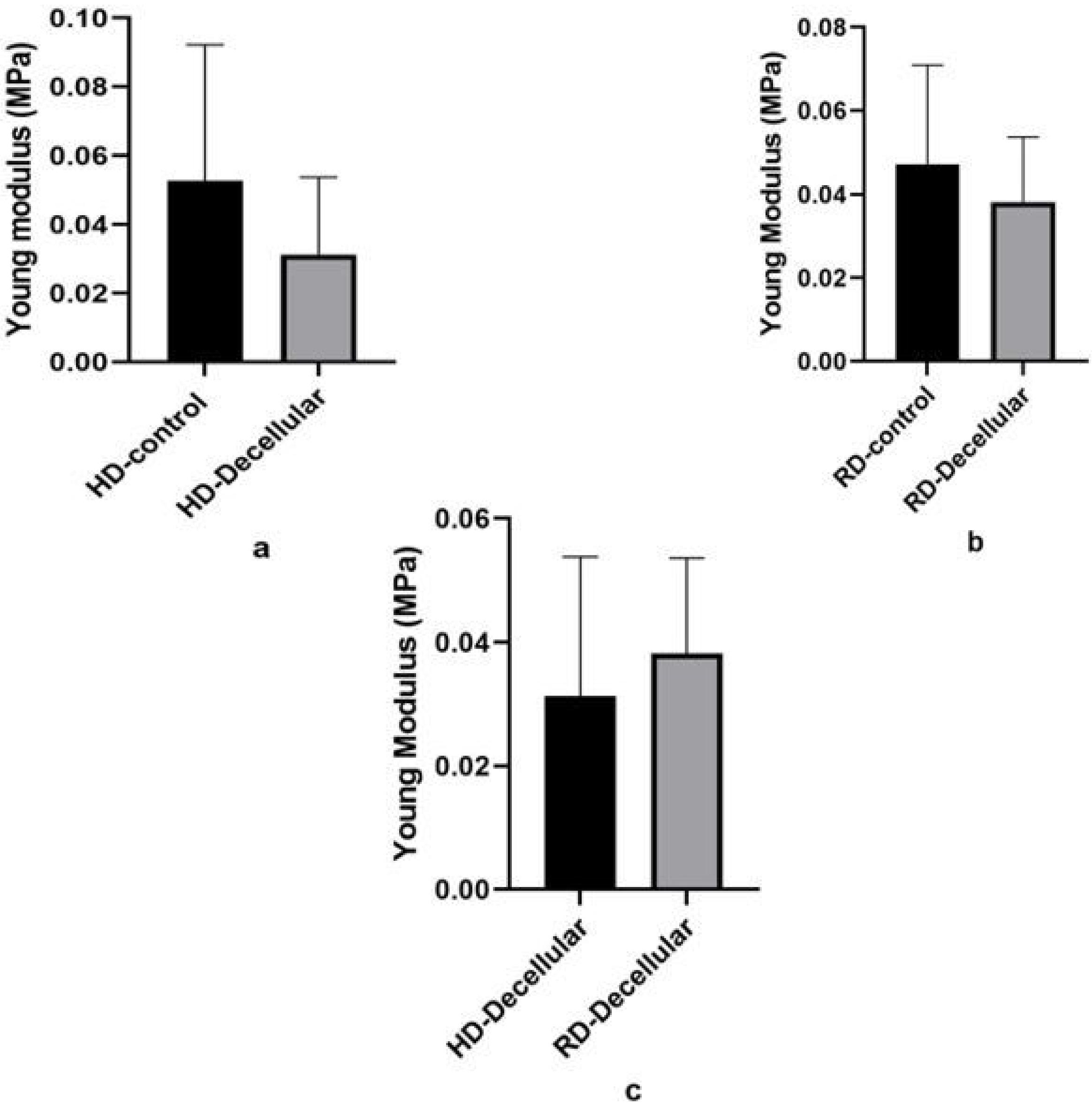

Figure 3

Comparison of mean Young modulus in rabbit skin groups (control and decellular) and human (control and decellular). a. The results showed that the mean elasticity in the human decellular skin group is lower than the control group, but this change is not significant ($p>0.05$). b. The results showed that the mean elasticity in the decellular skin group of rabbits is lower than the control group, but this change is not significant ($p>0.05$). c. The results showed that the mean elasticity is different in decellular derm of human and rabbit groups but this difference is not significant ($p>0.05$).