Journal of Biological Studies
ISSN 2209-2560

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JBS An Open Access Peer Reviewed Journal

Research Full-Text Paper

Healing effect of decellularized human placenta and gelatin hydrogel on diabetic wound in animal model

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Received: April 18, 2022; **Accepted:** May 4, 2022; **Published online:** August 30, 2022

Abstract: Diabetic skin wounds are the most critical clinical issues that are mostly resistant to usual treatments and healing methods. The aim of this study was to investigate the healing effect of decellularized human placenta and gelatin hydrogel on diabetic wound in animal model. Dorsal-paired 8–10 mm diameter wounds were created in the male Wistar diabetic rats. The rats were divided into control untreated group and groups treated with gelatin, decellularized placenta, and "decellularized placenta + gelatin". Macroscopic examination and wound area measurement were applied on day 0, and 7, 14 and 21 days after treatment to evaluate wound healing. The data were analyzed using ANOVA. Wound area significantly decreased 7 days after treatment in gelatin, decellularized placenta and "decellularized placenta + gelatin" treated rats compared to control animals, however, there was no significant difference in wound area between gelatin, decellularized placenta and "decellularized placenta + gelatin" groups. There was no also significant difference between the groups 14 and 21 days after treatment. Conclusively, our findings show that the decellularized placenta has healing properties on diabetic skin wound comparable to gelatin hydrogel.

Keywords: Decllularized placenta, Gelatin, Skin wound, Rat

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1 Introduction

Diabetes is one of the most common chronic metabolic diseases, affecting 371 million people worldwide (Davidson et al., 2003). The prevalence of diabetes in women in 2019 is estimated at 9.0% and in men 9.6% (based on age group). The prevalence of diabetes is directly related to income and development of countries, so that the prevalence of diabetes among high-income countries is relatively low. Revenues are higher. (Saeedi et al., 2019). But in general, research suggests that the prevalence of diabetes is approximately the same in men and women (Saeedi et al., 2019; Guariguata et al., 2014; Roglic, 2009; King et al., 1993), however, it has been reported that type 2 diabetes is more common in men (Nordström et al., 2016). Diabetes has several physical effects on a person, many of which are very serious and have devastating effects on a person's health, including diabetic wounds. If left untreated, diabetic wound can lead to amputation (Dokken, 2008; Kerr et al., 2019; Tang et al., 2013). Diabetic wounds are resistant to healing due to the involvement of active matrix metalloproteinases (MMPs). They are also characterized by poor recovery, decreased collagen production, and abnormal angiogenesis (Gooyit et al., 2014; Patel et al., 2019), so early detection, effective treatment, and accelerated healing of diabetic wounds are important (Brem et al., 2004; Mittal et al., 2020).

Decellularized tissue (or extracellular matrix (ECM)) is one of the most important factors that are effective in the healing of diabetic wounds (Han and Ceilley, 2017). Research suggests hyaluronan-derived components of ECM with anti-inflammatory activity play a significant role in the wound healing process (Chang, 2016). Studies have shown that in non-diabetic wound healing, molecular factors such as extracellular matrix are effective (Qing, 2017). Other studies have shown that ECM is involved in the angiogenesis of damaged tissues (Zhang et al., 2020). Further laboratory studies have shown that the use of ECM, with collagen deposition, improves diabetic ulcers (Agren and Werthen, 2007). Decellularized placenta has been reported to accelerate the healing of diabetic wounds by transmitting key signals for cellular processes (Schultz and Wysocki, 2009; Wang et al., 2019), however, the results are somehow ambiguous.

Despite all the efforts made in the field of diabetic wound healing, promising therapeutic results have not yet been achieved. Few studies have been carried out to demonstrate the healing effects of decellularized placenta on diabetic skin wounds. The aim of the present study was to investigate the healing effects of decellularized human placenta and gelatin hydrogel on diabetic wound in animal model.

2 Materials and Methods

2. 1. Preparation of the gelatin hydrogel

Aqueous gelatin solutions were prepared at 7%, 10% and 14% concentrations and added drop wise into emulsion bath including glutaraldehyde (GA) as crosslinker and oil. This bath was prepared using 20 mL of oil and 5 mL of GA solution. The mixture was gently stirred with a speed of 60 rpm for 1h. The formed beads were collected by filtration and immersed in distilled water in order to remove unparticipated ingredients. After purification, the beads were dried in oven at 40 °C (Pal et al., 2007).

2. 2. Preparation of decellularized placenta

The placenta was washed several times with distilled water to remove blood components. Distilled water was added to the placenta and the tissue / water mixture (2:1) was homogenized for 5 minutes at room temperature using a blender (Shinil Industrial Company). Placenta extract (ECM) was centrifuged at 3000 g for 5 minutes and the top layer containing blood residues was discarded. The ECM suspension was washed several times and centrifuged for 5 minutes. Subsequently, the ECM suspension was treated with 0.5% sodium dodecyl sulfate (SDS; Sigma) (diluted 1:1) for 30 minutes at room temperature in a vibrating water bath (Taitec). It was then centrifuged and washed with distilled water at room temperature for 4 days under shaking until the remaining SDS was removed. The suspension was then mixed with a mixture of 0.2% DNase (2000 U; Sigma) and 200 ug/mL RNase (Sigma) and was treated for 10 minutes at 37 °C. The final products were centrifuged and thoroughly washed with distilled water for 2 days. The final ECM and distilled water were mixed homogeneously in a ratio of 2:1 (v/v) and then the decellularized ECM was gently poured into a round mold, frozen at -70 ° C and dried in freezing. For at least 48 hours the de-cellulosic ECM sheets (15 mm diameter and 10 mm thick) were sterilized by ethylene oxide gas and hydrated with 10 ml saline phosphate buffer for 5 minutes before implantation (Li et al., 2019; Ulubayram et al., 2001). Decellularized placenta samples were confirmed by electron microscopy.

2. 3. Animal experiments

Male Wistar rats weighing between 200-220 g were purchased from Pasteur institute (Tehran, Iran). Food and water were freely available to the animals. Animals were kept on a 12 h light/dark cycle which would start at 8 am in the temperature range of 20-26 °C for one week. The rats were divided into 4 groups (n = 6 in each group): control untreated group and groups treated with gelatin, decellularized placenta, and "decellularized placenta + gelatin". Diabetes was induced by one injection of Streptozotocin (STZ, 40 mg/kg, Aladdin) into peritoneum. After one week of injection, the rats with fasting blood glucose higher than 16.7 mmol/L were considered diabetic.

The animals were anesthetized by intraperitoneal injection of 2 mg ketamine and 0.2 mg xylazine and their backs were moistened and completely shaved. The shaved area was disinfected with chlorhexidine gluconate. Dorsal-paired 8–10 mm diameter wounds were created in the rats using a sterile, disposable 6 mm biopsy punch tool. The dressings were fixed on the wound using Vaseline gauze and transparent adhesive tape. Images of burn wound area were captured on days 0,7,14 and 21 after wound establishment using a digital camera (S9+, Samsung, south Korea). The wound area was analyzed used using Image J software (Version 1.50i) (Kuo et al., 2016).

2. 11. Statistical analysis

All data were expressed as means ± SD and analyzed by one-way ANOVA ((SPSS® (version 20.0; IBM)). Difference between groups was analyzed by Tukey test and *P*-values less than 0.05 was considered statistically significant.

3 Results and Discussions

3. 1. Decellularized placenta sample

Placenta samples after decellularization was examined by electron microscopy confirming that the samples were decellularized successfully (Figure 1).

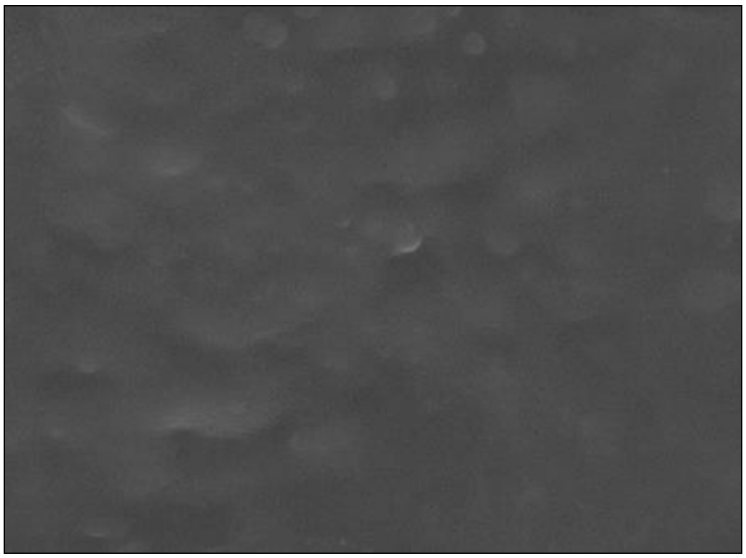

Figure 1: Electron microscope image of decellularized placenta.

3. 2. Wound closure

Macroscopic observation showed accelerated wound healing in gelatin + decellularized placenta treated group compared to other groups 21 days after treatment (Figures 2A, 2B, 2C, and 2D).

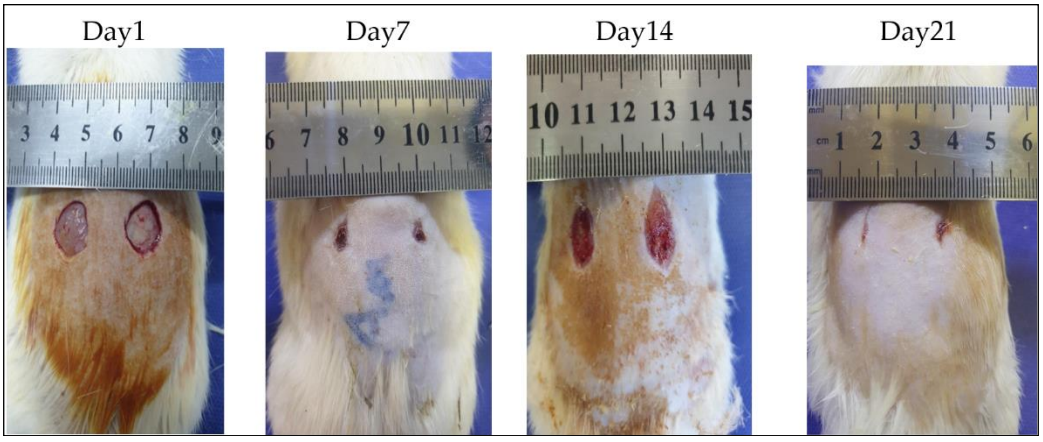

Figure 2A: Wound closure in the control group.

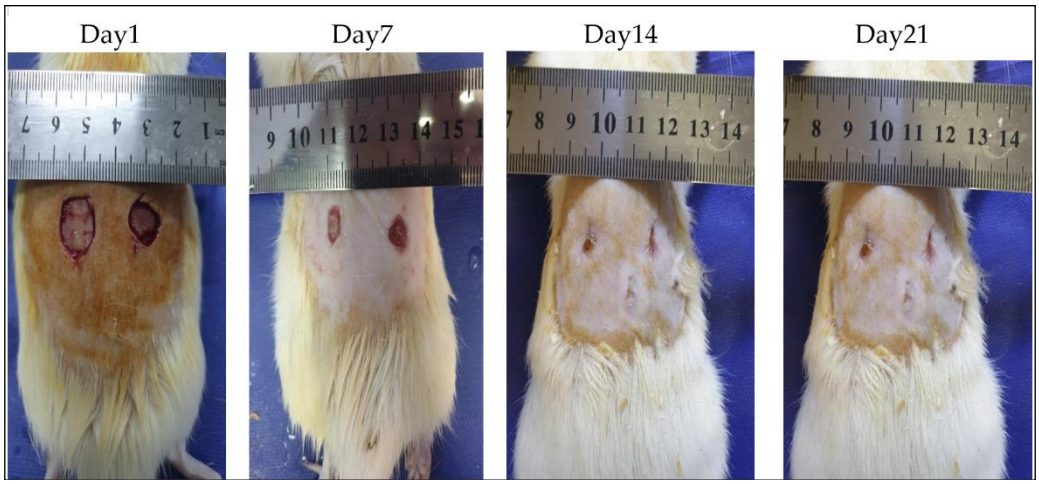

Figure 2B: Wound closure in the decellularized placenta group.

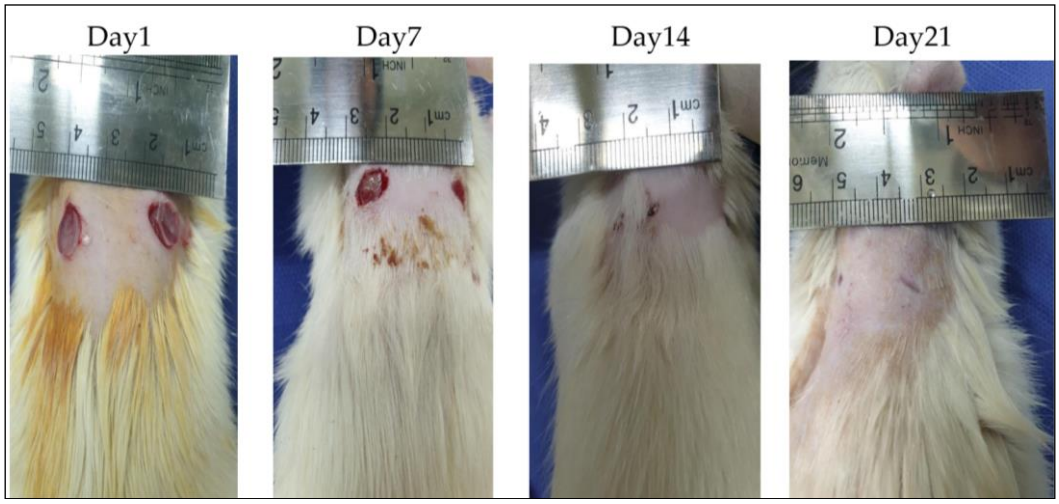

Figure 2C: Wound closure in the gelatin treated group.

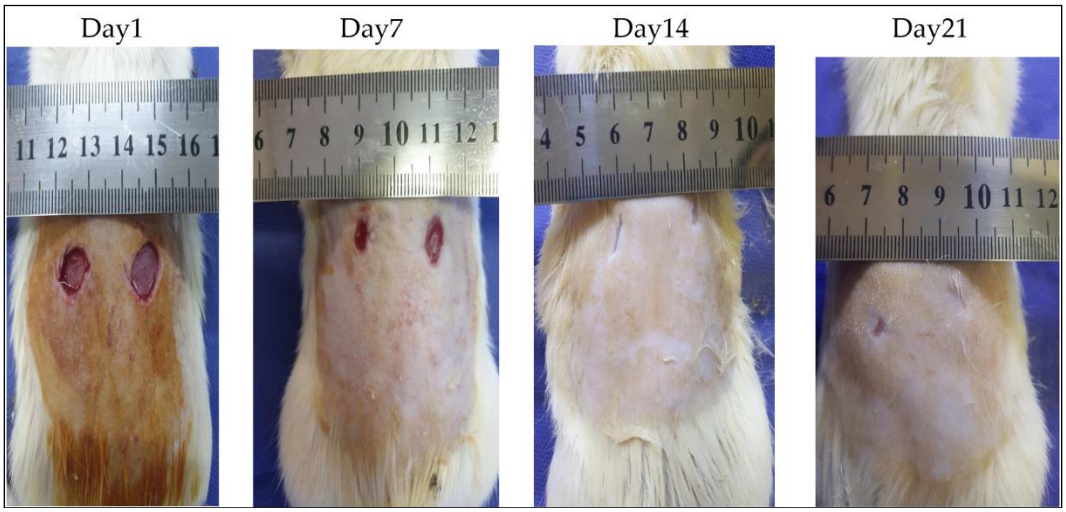

Figure 2D: Wound closure in the "gelatin + decellularized placenta" treated group.

3. 1. Wound closure

Our findings show that there was no significant difference in wound area between the control group and other groups on day 0. On day 7, a significant decrease was observed in the groups treated with decellularized placenta, gelatin and "decellularized placenta + gelatin" compared to the control group ($p < 0.05$, $p < 0.01$, and $p < 0.01$, respectively; however, no significant difference was observed between the group treated with gelatin and the group treated with "decellularized + gelatin". On day 14, no significant difference was observed between the control group and the group treated with decellularized placenta. However, a significant decrease in wound area was observed in the gelatin group compared to the control group ($p<0.01$). No significant difference in wound area was observed in the group treated with "decellularized placenta + gelatin" compared to the group treated with decellularized placenta. On day 21, no significant difference was observed between the control groups and other groups (Table 1 and Figure1).

Table 1. Wound skin area in the experimental groups on days 0, 7, 14 and 21.

Days	Groups	Mean±SEM	Mean±S.d	P1	P2	P3
Day 0	Control	0.934±0.016	0.934±0.029	-	-	-
	Decellularized placenta	1.046±0.129	1.046±0.223	N.S	-	-
	Gelatin	0.494±0.029	0.494±0.051	$P < 0.05$*	$P < 0.01$##	-
	Decellularized placenta + gelatin	0.611±0.049	0.611±0.085	$P < 0.05$*	$P < 0.05$#	N.S
Day 7	Control	0.688±0.088	0.688±0.153	-	-	-
	Decellularized placenta	0.386±0.019	0.386±0.033	$P < 0.05$*	-	-
	Gelatin	0.218±0.068	0.218±0.118	$P < 0.01$**	N.S	-
	Decellularized placenta + Gelatin	0.280±0.049	0.280±0.085	$P < 0.01$**	N.S	N.S
Day 14	Control	0.113±0.010	0.113±0.017	-	-	-
	Decellularized placenta	0.111±0.010	0.111±0.018	N.S	-	-
	Gelatin	0.029±0.002	0.029±0.003	$P < 0.01$**	$P < 0.01$##	-
	Decellularized placenta + gelatin	0.079±0.021	0.079±0.037	N.S	N.S	N.S
Day 21	Control	0.057±0.020	0.057±0.036	-	-	-
	Decellularized placenta	0.019±0.004	0.019±0.008	N.S	-	-
	Gelatin	0.018±0.005	0.018±0.009	N.S	N.S	-
	Decellularized placenta + gelatin	0.039±0.014	0.039±0.025	N.S	N.S	N.S

N.S indicates no significant difference. P1 indicates significant difference compared with control group, P2 indicates significant difference compared with decellularized placenta group, P3 indicates significant difference compared with gelatin group. (* indicates significant difference compared with control group and # indicates significant difference compared with decellularized placenta group).

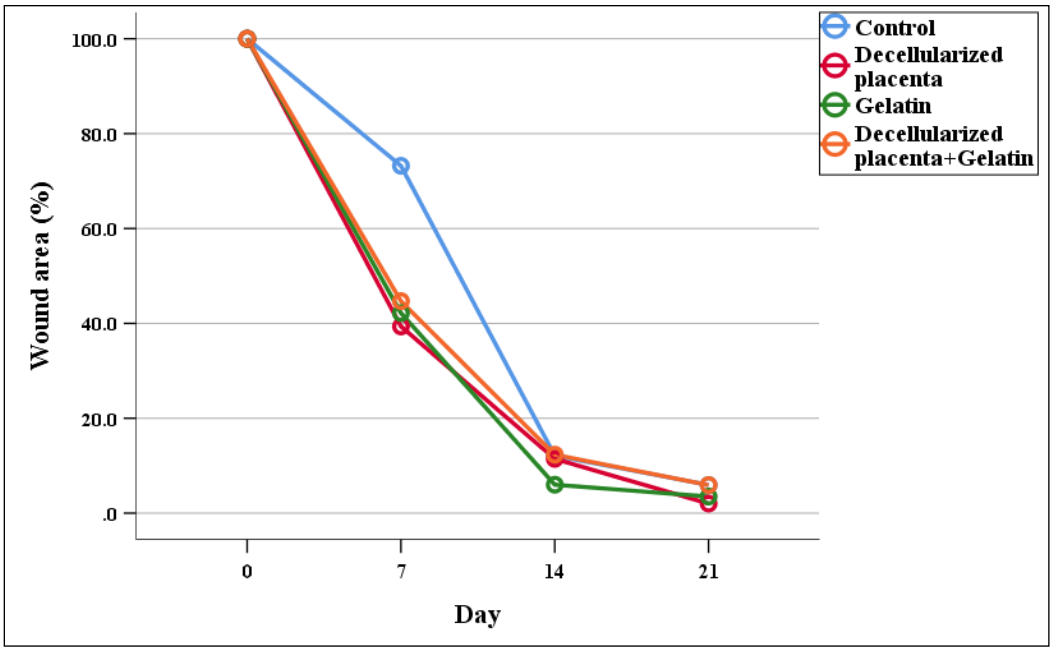

Figure 1: Percentage of wound skin area (%) in the experimental groups on days 0, 7, 14 and 21.

Perinatal-related tissues, such as the placenta, are generally discarded after delivery, however, they can be considered as alternative sources for decellularized extracellular matrix isolation. Although recent investigations have evaluated the efficacy of decellularized placenta in wound healing, there is still a need for more research assessing the effects of decellularized placenta on wound healing process due to challenging results obtained from previous and recent research on efficacy of decellularized placenta on wound healing. The finding of this study show that the decellularized placenta has healing properties on diabetic skin wound comparable to gelatin hydrogel. However, adding gelatin hydrogel to decellularized placenta could not increase the efficacy of each material on wound healing and did not show synergistic effects. In line with our findings, it has been shown that the decellularized tissue (extracellular matrix) has healing effects in injured and ulcerated mice (Livant et al., 2000). Previous studies have shown that decellularized tissues contain macromolecules (collagen, elastin, glycosaminoglycans, proteoglycans and connective tissue glycoproteins) that are able to regulate many important cellular functions, such as replication, migration, synthesis or degradation of proteins enabling them to play an important role in the wound healing process (Maquart and Monboisse, 2014). It has also been reported that matrix molecules regulate cellular functions, mediate cell-cell and cell-matrix interactions, and act as a reservoir and modulator of cytokines and growth factor function (Olczyk et al., 2014) which have a significant part in wound healing. On the other hand, defective extracellular matrix is associated with poor regeneration capability (Olaso et al., 2011). The results have revealed that placenta-derived extracellular matrix hydrogel has higher contents of chondroitin sulfate and heparan sulfate. In addition, molecular imaging showed that the placenta-derived extracellular matrix hydrogel exerted the best anti-inflammatory and proangiogenic effects in the skin wound healing model (Wang et al., 2020).

4 Conclusion

Overall, the results of this study show that the decellularized placenta has healing properties on diabetic skin wound comparable to gelatin hydrogel. Adding gelatin hydrogel to decellularized placenta could not increase the healing efficacy and did not show synergistic effects.

Acknowledgments

This research was supported by International Association of Scientists (IAS). We are thankful to the Stem Cell and Skin Research Center staff for their help and support.

Conflict of interests

The authors state that there are no conflict of interests regarding the publication of this article.

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