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Can Acellular Dermal Matrix and Kombucha Cellulose Membrane Be Used in Calvarial Reconstruction?

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ABSTRACT

Objective: Our objective was to study the applicability of acellular dermal matrix (ADM) and Kombucha membrane (KM) in repairing calvarial dura mater defects.

Methods: In a study conducted on Wistar rats, six groups were formed. A dorsal calvarial subperiosteal pocket was created in all rats. Two groups were implanted with an ADM and two groups with a KM. The other two groups were designated as control groups. Half of the six groups were sacrificed on day 30, while the other half were sacrificed on day 60. Tissue grafts were removed from the subperiosteal pocket and subjected to histological analysis. Neovascularization and calcification intensity were examined by preparing samples with hematoxylin-eosin and periodic acid Schiff stains and observing them under a light microscope.

Results: In the control group, intense calcification was observed, but neovascularization was not observed. In the KM group, intense calcification was observed in almost all animals in both the 30-day and 60-day samples, and while neovascularization was absent in the 30-day animals, signs of neovascularization were present in half of the 60-day animals. In the ADM group, while no signs of neovascularization were observed in the 30-day and 60-day tissues, calcification was seen in more than half of the 30-day animals and disappeared in the 60-day animals.

Conclusion: This study demonstrates the potential use of KM in calvarial reconstruction.

Keywords: Acellular dermal matrix, kombucha, calvarial reconstruction

INTRODUCTION

The reconstructive surgery of the calvarial tissue is as old as the history of brain surgery. After many successful and unsuccessful experiences over the years, this problem is now being attempted to be solved with various methods (1-4). The search for solutions that cause less damage to the tissue and provide better adaptation always leads to ongoing research in this field (2,5). Today, the goal of reconstructing bone defects is to close the defect with a material that does not create a foreign body reaction, is not resorbed in the late period, provides the required volume as close as possible, without creating functional loss in another part of the body, and can be obtained at the lowest possible cost, as quickly as possible.

One of these materials is acellular dermal matrix (ADM) grafts. The initial clinical studies on the use of ADM started with the

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restoration of full-thickness skin defects due to burns (6,7). After the successful results in this area, the areas of graft usage expanded rapidly, especially in periodontology (8,9), plastic and reconstructive surgery (10,11), neurosurgery (12,13), hand surgery (14), and otolaryngology (15) for various purposes. ADM is a dermal graft devoid of the epidermis and all other cellular elements to prevent tissue rejection and graft failure (16). They are prepared using the de-cellularization process of human cadaver donor skin (allogeneic) or mammalian skin donor sources (xenogeneic) natural dermal tissue (17). This reduces the need for autologous grafts and eliminates problems with donor site wounds (17).

Another reconstructive material is kombucha, a drink made by fermenting tea and sugar with a symbiotic culture of bacteria and yeast (SCOBY) (18) typically for 7-10 days (19). SCOBY is a biofilm of microorganisms that resembles a mushroom head (19). The popularity of kombucha has increased due to its anti-inflammatory potential, antioxidant activity, lowering of cholesterol levels and blood pressure, reduction in cancer spread, and improvement in gastrointestinal, liver, immune system, and function (20-23).

In this study, we investigated the effects of ADM and kombucha on mouse calvarium and thus their usability in human calvarium.

METHODS

Permission was obtained from the İstanbul University Animal Experiments Local Ethics Committee (decision no: 2013/105, date: 30.09.2013) for the study of Wistar rats. Six groups were created. A sagittal incision of 2 cm in the midline was made on the dorsal calvarial skin of all rats. After entering through this incision, the periosteum was first reached, and then a periosteal incision was made at the projection of the same incision under the microscope, after the periosteum was visualized. Using a periosteal elevator, the bone under the periosteum was separated to create a subperiosteal pocket (Figure 1). In two groups, a 0.5x1 cm piece of ADM graft was cut and placed into the subperiosteal pocket. A new three-dimensional pig-derived ADM composed of natural type I and III collagens (Mucoderm, botiss gmbh, Berlin, Germany) was used (Figure 2) (24). In the other two groups, a 0.5x1 cm piece of natural cellulose membrane obtained by Kombucha fermentation and sterilized with UV was placed into the subperiosteal pocket. "Kombucha" is a beverage made by fermenting tea and sugar with a SCOBY for 7-10 days, and a cellulose biofilm membrane is formed on the top of the beverage by extended fermentation (18). These membranes were dried and sterilized with UV light and used as grafts. The remaining two groups were designated as control groups, and only subperiosteal pockets were created without graft tissue implantation. Half of the six groups (Table 1) were sacrificed on the 30th day and the other half were sacrificed on the 60th day. Tissue grafts taken from the subperiosteal pocket were removed and sent for histological analysis. They were examined under a light microscope after being prepared with hematoxylin-eosin and periodic acid Schiff stains. Histological examination was performed using cross sections taken in the medial-lateral plane along the long axis of the graft to determine histological changes. They were visualized under low- and high-power microscopes to evaluate their macroscopic and microscopic appearances. The presence of microcalcifications or neovascularization was examined. For calcification, values such as '-' for none, '+' for low density, and '++' for high density were assigned based on the density of osteoid formation and microcalcification. For

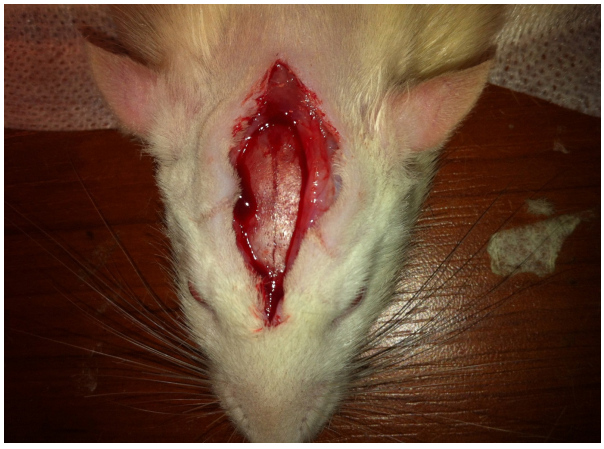

Figure 1. Creation of subperiosteal pocket

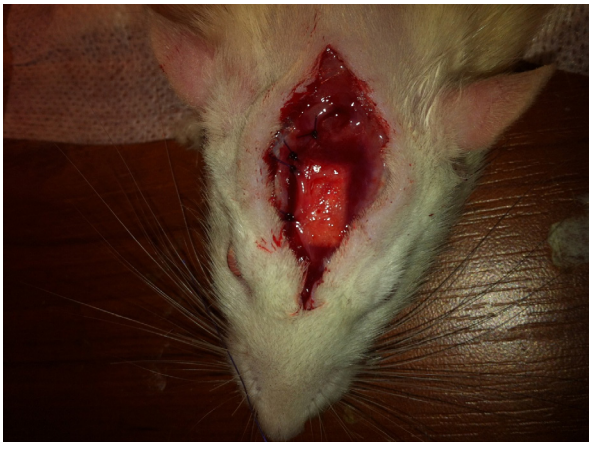

Figure 2. Implantation and detection of acellular dermal matrix

Table 1. Characteristics of the experimental groups

Group	Characteristics
Group-1	Control group, sacrificed on day 30
Group-2	Control group, sacrificed on day 60
Group-3	Implanted acellular dermal matrix, sacrificed on day 30
Group-4	Implanted acellular dermal matrix, sacrificed on day 60
Group-5	Kombucha cellulose membrane implanted, sacrificed on day 30
Group-6	Kombucha cellulose membrane implanted, sacrificed on day 60

neovascularization, values such as '-' for none, '+' for low density, and '++' for high density were assigned based on the density of lymphocyte infiltration and chronic inflammation.

Statistical Analysis

Statistical analysis was not performed in the study. Microscopic data was evaluated.

RESULTS

After completing the sacrifice process on days 30 and 60, calvarial tissue samples were taken from 6 groups and sent for histological examination. No infection or malignancy was found in the 48 subjects. When the results of the control group at 30 and 60 days were examined, intense calcification was observed but no neovascularization was seen. In the Kombucha membrane (KM) group, intense calcification was observed in almost all the tissues of both 30-day and 60-day animals. When KM was examined for neovascularization, no neovascularization findings were found in the 30-day subjects, whereas signs of neovascularization were present in half of the 60-day subjects. In the ADM group, no signs of neovascularization were observed in the 30-day and 60-day tissues. While calcification was observed in more than half of the 30-day subjects, it was observed that they disappeared in the 60-day subjects (Table 2) (Figures 3, 4, 5, 6).

DISCUSSION

In our study, we applied ADM and KM grafts to the calvarium of experimental animals to investigate their suitability for human calvaria. The two primary factors we investigated for suitability were neovascularization and calcification. Neovascularization was evaluated by assessing the presence of adequate scaffold function that allowed for re-epithelialization, neoangiogenesis, cellular

Table 2. Histological sections prepared from bone tissues left to heal without any treatment on their periosteums (control groups) and bone tissues left to heal by placing acellular dermal matrix and kombucha cellulose natural membrane under the periosteum were examined under a light microscope after 30-60 days. The sections were evaluated for lymphocyte infiltration and calcification

	30-day treatment		60-day treatment	
Control	**Neovascularization**	**Calcification**	**Neovascularization**	**Calcification**
1 Rat	-	++	-	++
2 Rat	-	++	-	++
3 Rat	-	++	-	++
4 Rat	-	++	-	++
5 Rat	-	++	-	++
5 Rat	-	++	-	++
7 Rat	-	++	-	++
8 Rat	-	++	-	++
Kombucha				
1 Rat	-	++	++	++
2 Rat	-	++	-	-
3 Rat	-	+	-	++
4 Rat	-	++	+	++
5 Rat	-	++	-	++
6 Rat	-	++	++	++
7 Rat	-	++	-	++
8 Rat	-	++	-	++
Acellular dermal matrix				
1 Rat	-	-	-	-
2 Rat	-	+	-	-
3 Rat	-	-	-	-
4 Rat	-	+	-	-
5 Rat	-	++	-	-
6 Rat	-	-	-	-
7 Rat	-	++	-	-
8 Rat	-	++	-	+

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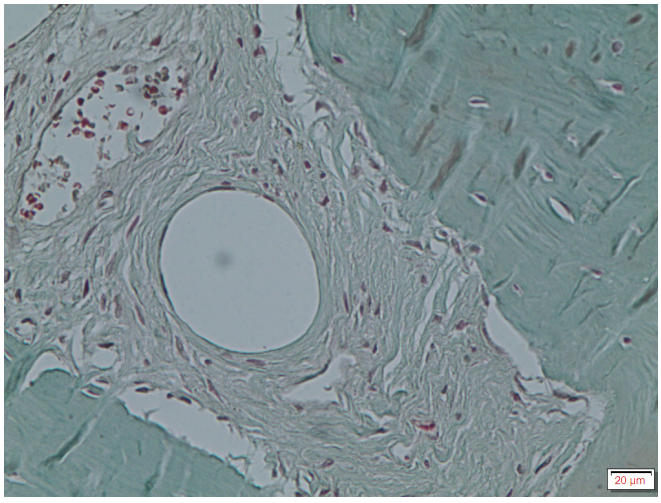

Figure 3a. In the control group, histological sections were prepared from the tissue left to heal and renewal stages were observed in the light microscopic images taken 30 days later. Hematoxylin-eosin staining was used

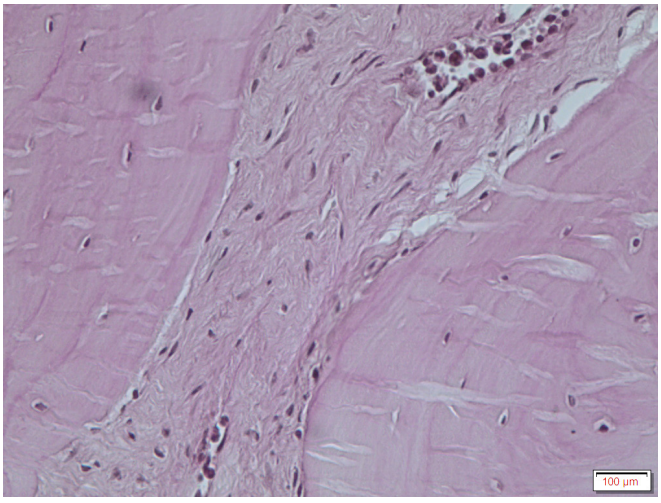

Figure 4. In the control group, histological sections were prepared from the healing tissue left to heal for 60 days and renewal stages were observed in the light microscopic images obtained. Hematoxylin-eosin stain was used

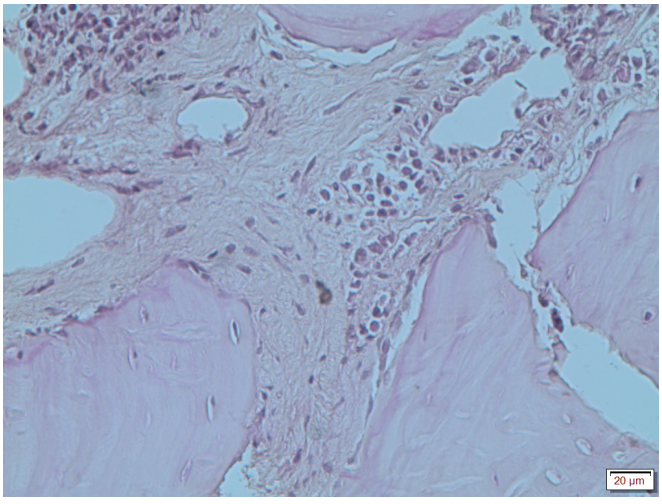

Figure 3b. In the control group, histological sections were prepared from the healing tissue left to recover and microscopic images were taken 30 days later, showing the stages of regeneration, using hematoxylin-eosin staining

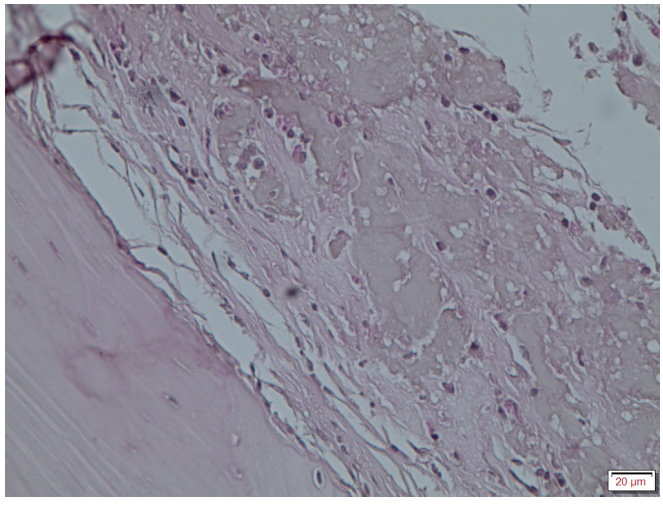

Figure 5. In histological sections prepared 30 days after implantation of acellular dermal matrix adjacent to the periosteum under the skin, light microscopic images showed cell-matrix interactions in the bone tissue. Periodic acid-Schiff staining was used

migration, and revascularization. Calcification was important for the restoration of the defect in the calvarial region for continued stability.

In our study, we found that KM had a strong calcification property, and signs of neovascularization began to appear in the late stage (60 days). However, we did not observe any neovascularization findings in ADM, and while there were signs of calcification at 30 days, these signs had disappeared at 60 days. These findings suggest that KM may be suitable for the calvarial defect reconstruction.

When looking at the literature, it is seen that many studies using ADM and KM have moved from animal experimental studies to studies on different tissues in humans with varying results. Studies on neovascularization and calcification with ADM and KM can only be seen in patients who undergo revision surgery. Our study allowed us to evaluate neovascularization and calcification results in a suitable number of experimental subjects. Revision surgery results are found only in a few case report series, and these results contradict each other (25,26). Additionally, studies have shown that ADM is useful in different areas, and there is less contraction

in wounds where it is used (27). Studies have also shown that ADM gradually replaces intact collagen fibers with host collagen, thereby supporting the healing process and minimizing the scar tissue formation (17).

KM has been used successfully in general surgical procedures to prevent adhesions, and it has also been shown to be an antimicrobial natural barrier (28-30). Although KM has various applications in modern medicine, no studies have been found in the literature on calcification and bone tissue reconstruction (31). In our results, we observed significant calcification and neovascularization began to develop after approximately 60 days.

Study Limitations

Although studies on experimental animals are one of the cornerstones of scientific research, it is difficult to make a clear decision without using the materials used in the study on humans. Apart from this, the number of test animals could be increased, or different materials could be trialed.

CONCLUSION

This study demonstrates the potential use of KM in calvarial reconstruction. Based on our promising and encouraging results, we are optimistic about the contribution of KM to calvarinal reconstruction. However, we believe that larger-scale experiments are needed to conduct clinical trials.

Ethics Committee Approval: Permission was obtained from the İstanbul University Animal Experiments Local Ethics Committee (decision no: 2013/105, date: 30.09.2013).

Informed Consent: Experimental study.

Peer-review: Externally and internally peer-reviewed.

Author Contributions: Surgical and Medical Practices - O.B., Ö.Ö., F.D.; Concept - O.B., A.B.; Design - O.B., A.B.; Data Collection and/or Processing - O.B., Ö.Ö., A.B.; Analysis and/or Interpretation - O.B., Ö.Ö., F.D., A.B.; Literature Search - Ö.Ö.; Writing - O.B., Ö.Ö.

Conflict of Interest: The authors have no conflict of interest to declare.

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