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Structural and Thermal Properties of Anisotropic Self-Inflated Sheep Skin and Palate

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Abstract: Structural and thermal properties of expanded tissue are different from those of non-expanded skin. In present research comparison of structural and thermal properties of palatal and skin tissue were investigated. Self-inflating tissue expander was placed beneath sheeps' palate and limb. After 3 weeks, the expander was removed. Five specimens each of expanded and control group were surgically harvested from the sheep. Thermal properties were measured using differential scanning calorimetric and thermogravimetric analysis, while structural morphology was investigated under scanning electron microscopy. Expansion with hydrogel caused the tissues to become oriented, ordered and denser. The thermal stability and glass transition temperature of expanded tissue were also higher than the control. The palatal tissue had higher thermal stability as compared to skin tissue. On the other hand, the skin tissue had higher glass transition temperature than the palatal tissue.

Keywords: *Self-inflating tissue expander; Expanded palatal and skin tissue; Thermal degradation; Structural architecture*

Introduction: Tissue expansion in-situ enables additional tissue to be formed for use in surgical reconstruction. The expansion is accomplished by placing a device called as tissue expander beneath tissues such as skin and let the growing expander stretched the tissue by means of step expansion or continuous expansion [1]. Neumann first introduced the use of tissue expander in clinical field in 1957 by inserting rubber implant subcutaneously to reconstruct external ear deformity [2]. A conventional tissue expander consists of silicone pouches with injection ports, which was integrated in implant or connected by tubing. Step expansion was employed by injecting saline into the port periodically.

Structural properties are one of the main factors contributing its usefulness to any engineering and medical application. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) has been used in these past few years for characterizing the thermal behaviour of materials as they undergo physical and chemical changes during heating. These methods have been applied to the study of the denaturation processes, which occur in biological macromolecules, such as collagen [6-8]. In the past years, many studies have investigated the biochemical and histological effects [9-11] on the epidermis and dermis, as well as on the blood supply [12-14] the capsule formation [15] and clinical outcomes [16, 17] of the tissue expanded with the tissue expanders. The present research compares the relationship between the structural

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architecture and thermal properties of the control and expanded skin and palatal sheep tissue. To achieve this aim, the structural architecture of the control and expanded skin and palatal tissue were determined using SEM, whereas the thermal properties of the tissue were determined using the TGA and DSC.

Materials & Method:

Animal protocol: Adult male Dopper sheep of 2 years old and average weight of 40kg were used in this study. Five animals each of control and expanded group were used. All procedures were performed under general anesthesia using a cuffed endotracheal tube fasted prior to the surgery. Sheeps were injected with ketamine 10% (Pharmaniaga, Malaysia) and maintained with isoflurane concentration set at 5% where depth of anesthesia was monitored and adjusted accordingly. The surgical field was thoroughly washed with 0.015% chlorhexidinegluconate and 0.15% cetrimide solution. A subcutaneous pocket was created by blunt dissection in a tension-free manner. A gamma sterilized anisotropic self-inflating tissue expander of 20 mm diameter and 3 mm thickness was slid through and implanted within the preformed pocket in the dorsolateral region. The surgical wound was sutured with interrupted 5-0 vicryl (Ethicon Inc, Johnson & Johnson, UK). In addition, three sutures were tied posterior to the expander to prevent device displacement and to close dead space. All sheep were monitored for 3-4 weeks prior to euthanasia. Euthanasia was carried out using a pentobarbital. The expanded skin was surgically removed; snap frozen and stored at -20^{o}C for characterization.

Scanning Electron Microscopy: Scanning electron micrographs of control and expanded skin tissues were taken under a scanning electron microscope. The palatal and skin tissues were coated with gold for making them conductive before SEM examination. Micrographs were subsequently taken at magnifications of 2,500 and 50,000.

Differential Scanning Calorimetry and Thermogravimetric Analysis: In separate set of experiments, differential scanning calorimeter (DSC QA100, Research Instruments) and thermogravimetric analyzer (TGA QA 100, Research Instruments) were used to analyze thermal properties of the skin samples. In each case, small amount of both control and expanded tissues were put into hermetic pans. Prior to DSC scanning, the decomposition rate of skin samples was measured using TGA by ramping from 25 to 500^{o}C at a rate of 10^{o}C. For DSC, the measurement was carried out by conducting heat-cool-heat procedure on the samples. The sample was heated from 25 to 250^{o}C at a rate of 10^{o}C. In between the first and second heating, the sample was cold to 0^{o}C at a cooling rate of 10^{o}C. DSC results were analyzed using U Mann-Whitney since the sample size was low, n=10 and not normally distributed. The analysis was conducted to see whether there is a significant difference in glass transition temperature and enthalpy of water vaporization between control and expanded tissue.

Results and Discussion:

Structural Properties: Figure 1 shows the surface architecture of the control and expanded palatal and akin tissue obtained from SEM. Both palatal and skin expanded tissues were found to

be more oriented and ordered as compared to the control tissues. The expanded tissues were also denser and had smaller inter-tissue spacing than the control tissues. It can be said from the comparison of skin and palatal tissues, the skin tissues were more oriented and ordered than the palatal. Thus the skin tissues had higher density and smaller inter-tissue spacing than the palatal tissues.

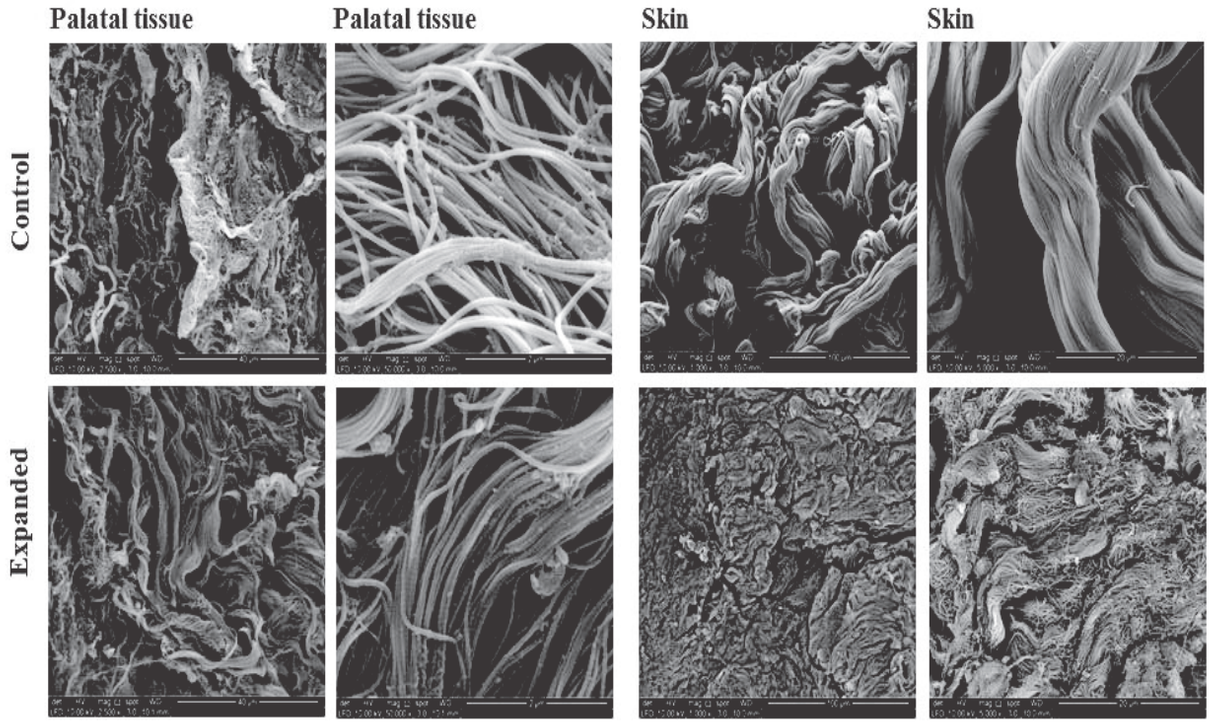

Fig. 1: SEM micrographs of control and expanded skin and palatal sheep tissues

Thermal Properties:

TGA Results: TGA graphs of normal and expanded palatal tissues are shown in Figure 2. Based from the Figure, it can be said that the control palatal decomposed at a temperature of 292^{o}C, while the expanded tissue decomposed at a temperature of 328^{o}C. Besides at 500^{o}C, both control and expanded tissue degraded to below 10% of initial weight. On the other hand, residue percentages left at 500^{o}C for control and expanded tissue was 5% and 4% respectively (Table 1). TGA graphs of normal and expanded skin tissues are shown in Figure 2. The control skin decomposed at a temperature of 287^{o}C, while the expanded skin decomposed at a temperature of 295^{o}C. Besides, at 500^{o}C, both control and expanded skin degraded to below 30% of initial weight. The residue percentages left at 500^{o}C for control and expanded skin tissue was 25% and 18% respectively (Table 1). Thus the control palatal and skin tissue had almost same decomposition temperature; however the expanded palatal tissue had higher thermal stability as compared to the expanded skin tissue.

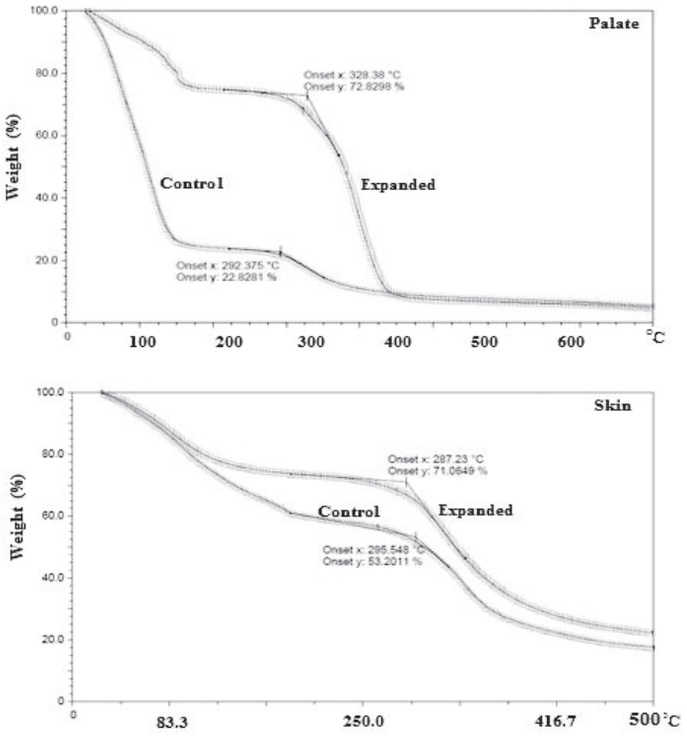

Fig. 2. TGA curves of control and expanded palatal and skin tissue

Table 1. TGA analysis results of control and expanded palatal and skin tissue

Specimen	Decomposition Temperature (^{o}C)	Residue left at 500^{o}C (%)
Control palatal tissue	292	5
Expanded palatal tissue	328	4
Control skin tissue	287	25
Expanded skin tissue	295	18

DSC Results: Table 2 shows the glass transition temperature and enthalpy of water vapourization for both control and expanded palatal tissue. The glass transition temperature of expanded palatal tissue was higher than that of control palatal tissue, while control tissue had higher enthalpy of water vapourization as compared to expanded tissue. The same phenomena happened with skin tissues (Table 2). The glass transition temperature of expanded skin tissue was higher than that of control skin tissue. On the other control tissue had higher enthalpy of water vapourization as compared to expanded tisuue. Thus the skin tissue had higher glass transition temperature and lower enthalpy of water vaporization as compared to the palatal tissue. The U Mann-Whitney analysis was conducted to see whether there is a significant difference in glass transition and enthalpy of water vaporization between control and expanded skin. Significance value was set at α=0.05. Based on the findings, both glass transition ($p<0.01$) and

enthalpy of water vaporization ($p<0.01$) showed significant result in palatal tissue. There is a significant difference in median of glass transition temperature between control palatal tissue and expanded palatal tissue. The expanded tissue showed higher median compared to the control skin.Based on the findings of skin tissue, only enthalpy of water vapourization showed significant result ($p<0.01$). There was a significant median difference in enthalpy of water vaporization between control skin and expanded skin. The control skin showed higher median compared to expanded skin. However, no significant result obtained for glass transition with $p=0.675$. There was no significant difference in median of glass transition between control skin and expanded skin.

Table 2. DSC analysis results of control and expanded palatal and skin tissue

	Median (IqR)		Z stat	P-value
Palate	Expended	Control		
Glass transition temperature (^{o}C)	175 (3.90)	170 (3.00)	-2.61	0.009
Enthalpy of water vaporization (J/g)	1465 (40.50)	1577 (35.00)	-2.61	0.009
Skin	Expended	Control		
Glass transition temperature (^{o}C)	191 (7.85)	182 (15.80)	-0.42	0.675
Enthalpy of water vaporization (J/g)	856 (8.50)	870 (14.00)	-2.62	0.009

Present research describes the changes in structural and thermal properties of expanded palatal and skin tissues of sheep that were expanded using anisotropic self-inflating hydrogel expander. TGA results of present research indicate that the thermal decomposition temperature of expanded palatal and skin tissues was higher as compared to the corresponding control tissue (Figure 2). This might be due to structural stability after expansion. According to DSC results, the glass transition temperature of expanded palatal and skin tissue was slightly higher than the corresponding control tissue (Table 2). This might due to orientation during and after expansion. Notably, the tissue became more oriented and denser after expansion. On the other hand, the enthalpy of water vapourization of control palatal and skin tissue was found higher than that of expanded tissue as the control tissue had higher water content as compared to expanded tissue (Table 2). The hydrogel expander might have removed some water from the tissue during expansion. When the thermal properties of palatal tissue were compared with skin tissue, it was found that the control palatal and skin tissue had almost similar thermal stability. However, the expanded palatal tissue had much higher thermal decomposition temperature as compared to the expanded skin tissue. The expander removed more water from the palatal tissue than it removed from the skin tissue. This might be the cause of higher thermal stability of the palatal tissue. On the other hand, the skin tissue had higher glass transition temperature as compared to the palatal tissue as the palatal tissue had higher water content than the skin tissue.

Conclusions: Comparison of the structural and thermal properties of normal and expanded palatal and skin tissue were investigated in present research. Self-inflating hydrogel tissue expander was placed beneath palate and limb of sheep. After 3-4 weeks, the expander was

removed. Both the palatal and skin tissues became oriented, ordered and denser after expansion. The expanded palatal and skin tissues also had slightly higher thermal stability and glass transition temperature as compared to the corresponding control skin tissue. The palatal tissue had higher decomposition temperature as compared to skin tissue. On the other hand, the skin tissue had higher glass transition temperature than the palatal tissue.

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