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Comparative Study on Physical Properties of Different Tissue-Derived Collagen Biomaterials

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Abstract
In this paper, physical properties of different tissue-derived collagen biomaterials were studied, including morphology, roughness, porosity, hydrophilicity, mechanical strength and denaturation temperature. Results demonstrated that physical property of porcine small intestinal submucosa extracellular matrix (VIDASIS) was almost the same with other domestic and foreign products.

Keywords
Collagen, Biomaterial, Mechanical Strength, Physical Property

1 Introduction
Collagen belongs to the structural protein of the extracellular matrix. It is the main component of the extracellular matrix. The relative molecular mass is about 300 kDa. It contains one or more regions with a triple helix structure composed of alpha chains. It is the most abundant in mammals (about 25%~30% of total protein), the most widely distributed functional protein. As a fibrous protein, collagen can be used as an excellent biomaterial for tissue engineering scaffolds [1] [2]. As a biomedical material, collagen has the characteristics of low immunogenicity, good biocompatibility, biodegradability, and hemostasis [3] [4] [5] [6] [7]. In addition, the triple helix structure and Its own cross-linked structure makes it have high strength, which can

meet the body's requirements for mechanical strength, and it has become an important role in tissue engineering.

The decellularized porcine small intestinal submucosal matrix is a membranous biomaterial prepared by decellularization, molding, and sterilization processes using the porcine small intestinal submucosa (SIS) as a raw material. Its main component is collagen and also contains fibronectin , Laminin, growth factor and other biologically active functional molecules. It has been widely used in the repair and reconstruction of tendons, dura mater, abdominal wall and other tissues. In addition, it has also been reported that collagen products prepared from bovine dermis, bovine Achilles tendon, and pig skin have been used in clinical treatments, and have good therapeutic effects[8]-[13]

This article compares and examines the physical properties of four collagen biomaterials: the porcine small intestinal submucosal matrix material (VIDASIS), the imported porcine small intestinal submucosal matrix material (BIODESIGN), and the commercially available acellular bovine dermal matrix (ADM) and collagen sponge (CS). , Including morphology, roughness, porosity, hydrophilicity, mechanical strength, and denaturation temperature, so as to have a more comprehensive grasp of the mechanical properties and physical properties of collagen biomaterials from different tissue sources, and provide reference value for the continuous improvement of product quality.

2. Experimental part

2.1. Main raw materials

Acellular porcine small intestinal submucosal matrix material (VIDASIS), a product of Beijing Biosis Healing Biological Technology Co., Ltd.; acellular porcine small intestinal submucosal matrix material (BIODESIGN), a product of COOK Biotech, USA; acellular dermal matrix is a product of bovine dermal tissue Samples prepared after decellularization process (AMD), products of Yantai Zhenghai Biotechnology Co., Ltd.; collagen sponges are cow heels extract collagen and freeze-dried samples (CS), Tianxinfu (Beijing) Medical Equipment Co., Ltd. product.

2.2. Main instruments

The main instruments used in this research are shown in Table 1.

Table 1. Mainly used instruments in this study

Instrument	Model	Manufacturer
scanning electron microscope	S4800	Hitachi
atomic force microscope	FastScan Bio	Bruker
True density meter	JW-M100A	Beijing Jingwei Gaobo Science and Technology Co., Ltd.
Video optical contact angle measuring instrument	DSA100	KRüSS

Pharmaceutical packaging performance tester	MED-1	Jinan Languang Electromechanical Technology Co., Ltd.
Differential Thermogravimetric Synchronous Analyzer	STA449 C	Germany Netzsch Instrument Manufacturing Co., Ltd.

2.3. Shape analysis

The pore size and fiber structure of the four samples were observed by scanning electron microscope. The samples were dried at 40°C for 24 hours, sprayed with gold by ion sputtering instrument, and observed by scanning electron microscope.

2.4. Surface roughness analysis

The roughness of the four groups of materials was detected by the FastScan Bio atomic force microscope of the German Bruker Analytical Instruments Company. The scanning tip adopts Φ0.5 mm high-purity tungsten, the bias voltage between the tip and the sample is 80 mV, the scanning range is 1750 nm × 1750 nm, and the image is processed by Nano-Scope Analysis software.

2.5. Porosity measurement

Weigh the mass M of the sample with a one-ten-thousandth balance, then measure the size of the sample with a vernier caliper and calculate its apparent volume V, then put the sample into the sample cup, and use the true density meter to measure the true density of the sample ρ true and the true of the sample If the volume V is true, use the following formula to calculate the porosity ε of the sample.

$$\varepsilon = [(1/\rho_{视}) - (1/\rho_{真})]/(1/\rho_{视})$$

Among them, $\rho_{视}$ is regarded as the apparent density, and $\rho_{视}$ is M/V;
$\rho_{真}$ is the true density, which is the measured value of the instrument

2.6. Hydrophilicity analysis

Place the four dried samples on a cover glass, drop a drop of water (about 2 μL) on the surface of the material, and measure the contact angle between the water droplet and the material with a video optical contact angle meter to measure the surface energy of the material.

2.7. Mechanical performance testing

The four materials were cut into samples with a width of 20 mm and a length of 30 mm. The distance between the clamps was 15 mm, and the samples were clamped to the clamps. The force, stress, strain and elastic modulus of the samples were measured by a medical packaging performance tester. quantity.

2.8. Differential thermal analysis

The four samples were crushed into powder, and the differential thermal and thermogravimetric analysis was performed on the samples using a differential thermogravimetric synchronous analyzer. The maximum temperature is 600°C, the heating rate is 10°C/min, and the atmosphere is air.

3. Results and discussion

3.1. Shape analysis

The surface and cross-sectional morphology of the four materials are shown in Figure

1. It can be seen from the figure that the collagen fibers are arranged in a crisscross pattern on the surface of the two SIS materials. The fibers on the material surface are obvious. The fiber diameter is about 1 μm. The fibers are well preserved, the length is above 50 μm, no obvious broken fibers are found, and there are communicating holes that lead into the material. The diameter of the SIS fiber can be seen from the cross-sectional topography. The two SIS materials have a layered structure and are evenly distributed. However, the VIDASIS layer gap is larger, which is more conducive to the growth of new cells. The surface of ADM is relatively flat and dense. There are few connected pores on the surface. There are many isolated lumps in the cross section. It is the cross section of extracellular matrix fibers. There are many internal connected pores. This material should be stacked with fibers in different directions. become. The surface of CS is a fibrous mesh structure. From the cross-sectional topography, it can be seen that the pore size of the material is different, and the pore connectivity is good. In general, the four materials have interconnected pores, but the surface of ADM is compact and flat, with a micron-scale sheet structure (with a length of more than 100 μm), which limits the growth of cells. The interconnected pores inside the BIODESIGN sample are relatively poor compared to other samples. VIDASIS and CS samples have the best connection effect. However, CS is prepared by freeze-drying process of collagen solution, so the pore interconnection structure is the best, and the pore size ranges from 50 to 200 μm.

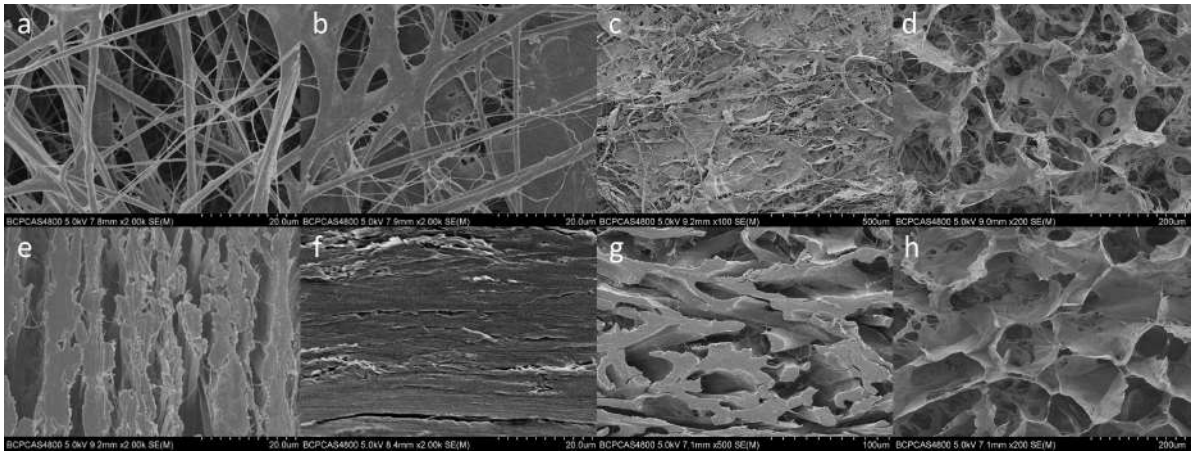


Figure 1. Images of scanning electron microscope of four biomaterial samples

(a: VIDASIS surface; b: BIODESIGN surface; c: ADM surface; d: CS surface; e: VIDASIS side; f: BIODESIGN side; g: ADM side; h: CS side)

3.2. Roughness analysis

The roughness of the sample was analyzed by atomic force microscope, and the results are shown in Table 2. The roughness of VIDASIS and BIODESIGN is relatively small. The roughness of ADM is obviously greater than that of SIS material. The Rq and Ra values are 224 nm and 185 nm, respectively, while the roughness of SIS material is below 100 nm. The surface of SIS is relatively flat, which is easier for cell migration and repair, thereby reducing the risk of adhesion. Because the roughness of CS is too large, it exceeds the test range of the atomic force probe, and the roughness cannot be measured.

3.3. Porosity measurement

Porosity is one of the criteria for characterizing tissue engineering scaffold materials. On the one hand, the pores of the scaffold are channels for cells to enter the scaffold; on the other hand, the pores of the scaffold are also important channels for nutrients, gases, and metabolites of implanted cells to enter and exit the scaffold. Both SIS materials have a certain porosity, which is conducive to the growth of cells and blood vessels, is conducive to tissue repair, reduces the degradation time of repair materials in the body, accelerates tissue repair and wound healing, and meets the requirements of tissue engineering scaffold materials. basic requirements.

As shown in Table 3, the porosities of VIDASIS, BIODESIGN, ADM and CS are 45.82%, 44.07%, 74.99% and 98.84%, respectively. The porosities of the two SIS materials are similar, both being about 45%. CS has the highest porosity. Due to the production process and other reasons, its product is porous sponge three-dimensional structure. The porosity of ADM is between SIS material and CS material.

3.4. Hydrophilicity test

As shown in Figure 2, the water contact angle of VIDASIS material is 45˚ ± 1.7˚, BIODESIGN material is 46˚ ± 2.1˚, ADM material is 102˚ ± 0˚, and CS is 96.5˚ ± 4.95˚. The water contact angle is an important measure of the wettability of the reacting substance and the liquid. The smaller the water contact angle, the better the hydrophilicity of the material. The water contact angles of the two SIS products are not significantly different and both are less than 90˚, indicating that the SIS materials produced by the two companies have good hydrophilicity. The water contact angles of ADM and CS are both greater than 90˚, and the materials are hydrophobic. The hydrophilic effect is good, which is good for body fluids to infiltrate the material and vascularization. It is good for cells to grow into the material, and gradually fill the material with new cells, promote the tissue repair process, and accelerate the repair and healing of the diseased area.

Table 2. Results of roughness analysis by atomic force microscope

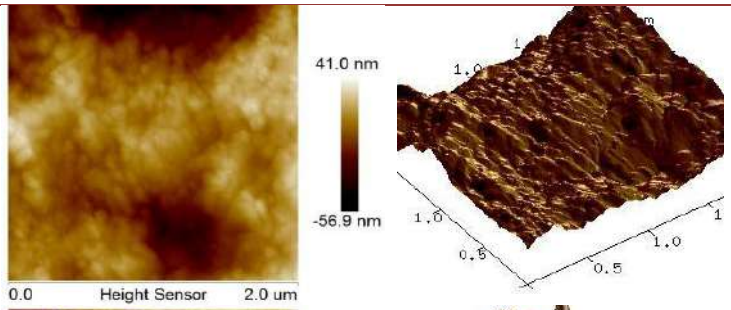
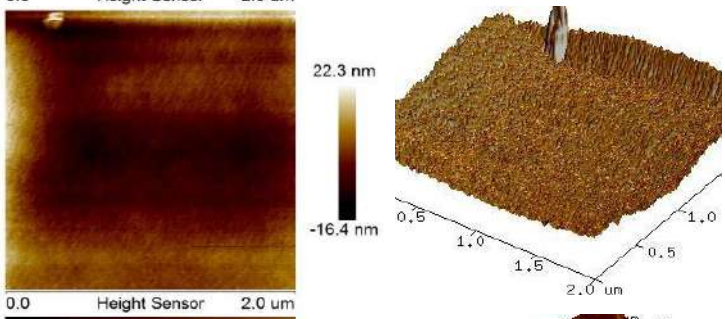
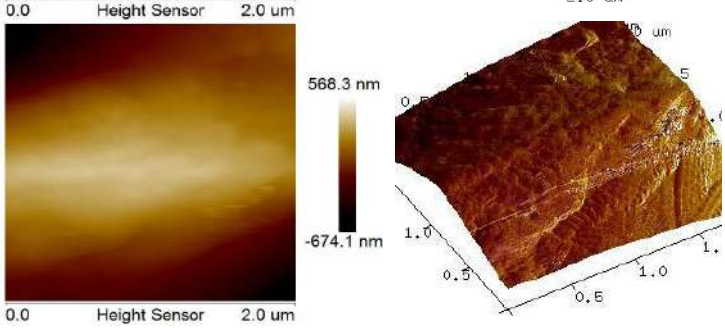
Sample	Atomic Force Microscope Image	Roughness	
VIDASIS		14.3	11.0
COOK		5.72	4.63
ADM		224	185
CS	The roughness of the sponge is too large, beyond the test range of AFM	-	-

Table 3. Results of porosity test of four biomaterial samples

SAMPLE	V/cm^3	ρ 视 /g·cm^{-3}	V 真 /cm^3	ρ 真 /g·cm^{-3}	ε/%
VIDASIS	1.2133	0.6110	0.6556	1.1304	45.82
BIODESIGN	1.5068	0.6404	0.8446	1.1426	44.07
ADM	0.3377	0.2810	0.0845	1.1292	74.99
CS	11.0864	0.0101	0.1281	0.8793	98.84

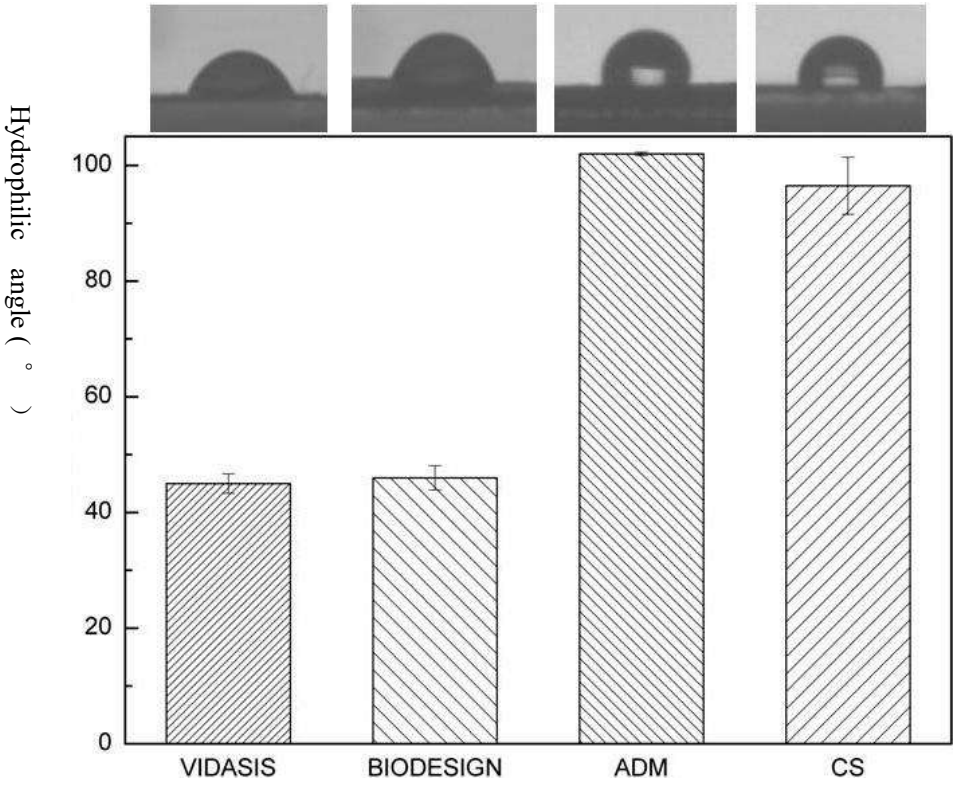


Figure 2. Images of hydrophilicity test of four biomaterial samples

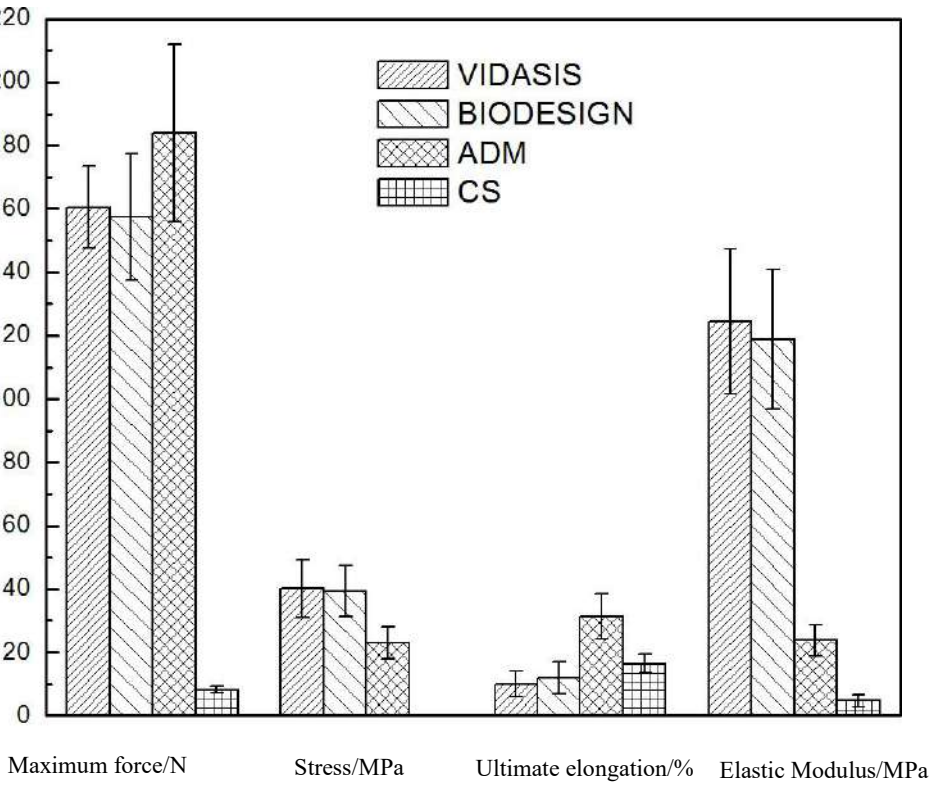
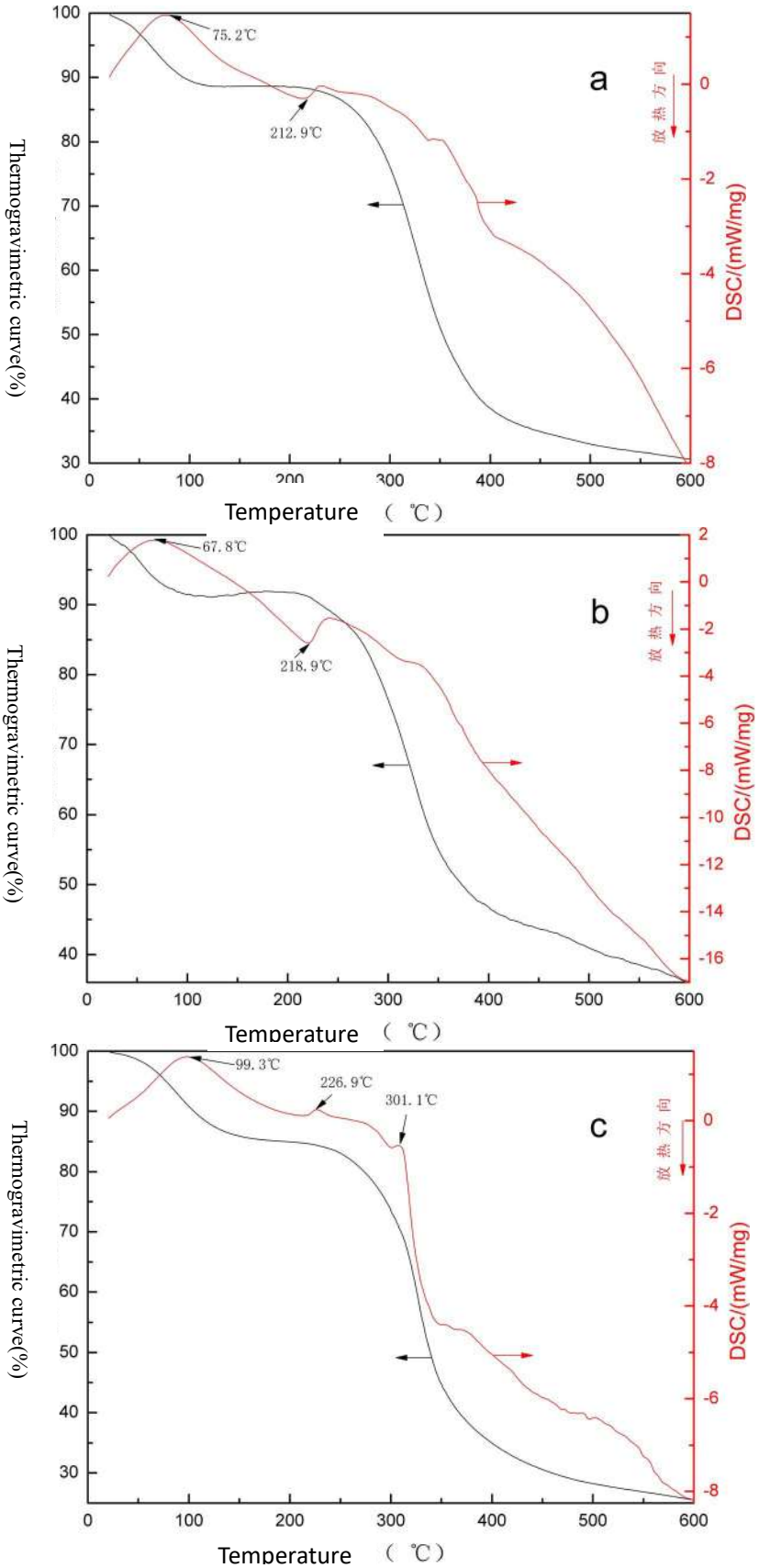


Figure 3. Results of mechanical properties of four biomaterial samples


a
75.2℃
212.9℃
放热方向
Thermogravimetric curve(%)
DSC/(mW/mg)
Temperature （℃）
b
67.8℃
218.9℃
放热方向
Thermogravimetric curve(%)
DSC/(mW/mg)
Temperature （℃）
c
99.3℃
226.9℃
301.1℃
放热方向
Thermogravimetric curve(%)
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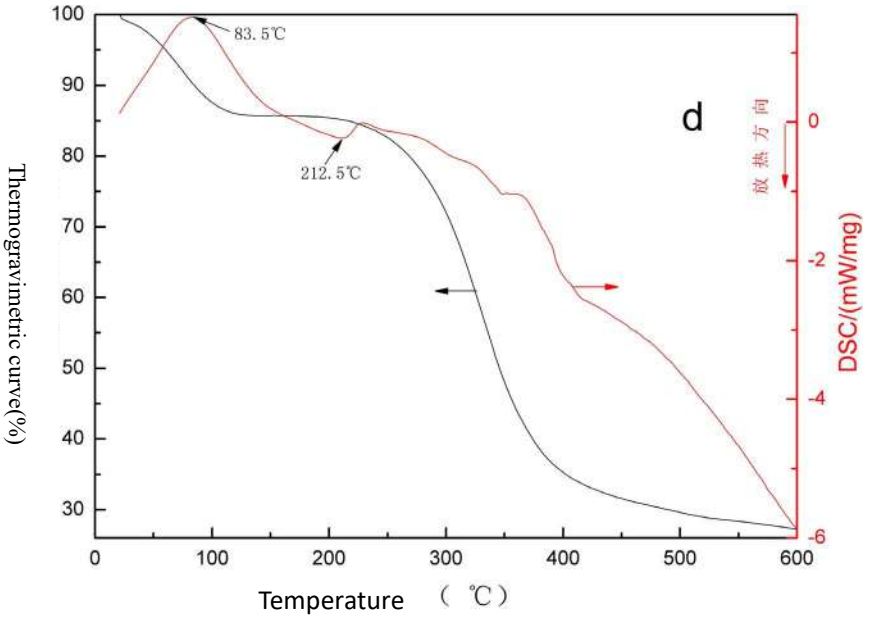

Figure 4. Results of differential scanning calorimetric test of four biomaterial samples (a: VIDASIS; b: BIODESIGN; c: ADM; d: CS)

3.5. Mechanical performance test

The mechanical performance test results are shown in Figure 3. VIDASIS maximum force value is 160.5 N, maximum stress is 40.1 MPa, maximum strain is 10%, elastic modulus is 124.43 MPa; BIODESIGN maximum force value is 157.5 N, maximum stress is 39.4 MPa, maximum strain is 12%, elastic modulus 118.69 MPa; the maximum force value of ADM is 184 N, the maximum stress is 23.0 MPa, the maximum strain is 31.3%, and the elastic modulus is 23.74 MPa; the maximum force value of CS is 8.2 N, the maximum stress is 0.072 MPa, and the maximum strain is 16.3%, the modulus of elasticity is 4.78 MPa.Comprehensive comparison, the mechanical properties of the two SIS materials are basically the same, and the elastic modulus is significantly better than the other two materials, and the mechanical properties of CS are poor. It is reported in the literature that the horizontal mechanical strength of the abdominal wall tissue is 10 ± 3.4 MPa, and the vertical mechanical strength is 4.5 ± 2.0 MPa [14]. Therefore, the mechanical strength of the two SIS materials can meet the requirements of abdominal wall repair, while the CS material is basically not due to poor mechanical properties. May be used for the repair of abdominal wall defects. In addition, from the comparison of the ultimate elongation of the material, the ADM material is more elastic, and its strain is more than three times that of the SIS material. After being implanted in the body, it is prone to shrinkage and deformation as the collagen is degraded and reshaped.

3.6. Differential thermal analysis

The differential thermal curve can reflect the phase change, decomposition, combination, solidification, dehydration, evaporation and other physical or chemical reactions of materials, so it can be used to analyze the denaturation temperature of collagen. As shown in Figure 4, the differential thermal curves of the four materials show that there is a heat absorption peak around 60 ℃ ~100 ℃ , this peak is the denaturation temperature of the material, that is, the temperature at which the triple

helix structure of collagen is destroyed. The denaturation temperatures of VIDASIS, BIODESIGN, ADM and CS are 75.2℃, 67.8℃, 99.3℃ and 83.5℃, respectively. ADM and CS are two The denaturation temperature of these materials is significantly higher than that of SIS materials. ADM may be related to the constraint of containing elastin, while the vacuum dehydration process of CS materials increases the denaturation temperature of collagen molecules. The second endothermic peak is around 220° C, and the difference between the four materials is different, which means that the collagen structure is completely destroyed.

The thermogravimetric curves of the four materials all show two weight loss processes. The first stage is from room temperature to 200° C. The weight loss rates of VIDASIS and BIODESIGN are both about 10%, while the weight loss rates of ADM and CS are higher, about 15%. This process is the desorption process of physically adsorbed water in the collagen sample; The second stage is from 200° C to 400° C. This process is mainly the thermal degradation process of collagen, and is also accompanied by other weight loss processes, such as carbonization and dry distillation. The weight loss after 450° C is more complicated, mainly due to its heat. Caused by carbonization of decomposition products[15]

4. Conclusion

This article examines the physical properties of four collagen biomaterials: VIDASIS, BIODESIGN, ADM and CS. Through comparative analysis, collagen biomaterials of different tissue sources and forms show differences in specific physical properties.

VIDASIS has a rougher surface than BIODESIGN. The former has a higher porosity than the latter. The roughness and porosity of CS is higher than the other three. ADM is between the three. VIDASIS and BIODESIGN have a smaller hydrophilic angle than ADM and CS, and are more hydrophilic, more easily wetted by body fluids, and can better contact the repaired tissue. VIDASIS and BIODESIGN have the highest mechanical strength, and the amount of strain is smaller than that of ADM and CS. Under normal circumstances, the chest pressure and abdominal pressure will increase when the human chest and abdomen muscles contract, especially during coughing, defecation, and heavy physical work. The pressure rise is particularly obvious. Repairing damaged abdominal wall materials requires a certain degree of mechanical strength. CS The tensile strength of SIS is low and cannot meet the requirements of use, while the tensile strength of SIS and ADM materials far exceeds the ultimate tensile strength of the abdominal wall, meeting the requirements for repairing abdominal wall damage.

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