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Comparative Study on Degradation Process of Acellular Porcine Small Intestinal Submucosa Matrix *In Vivo* and *In Vitro*

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Abstract

Degradation behaviors of acellular porcine small intestinal submucosa (SIS) matrix under differ- ent circumstances were investigated by taking the degradation experiments *in vivo* and *in vitro* respectively. *In vitro*, solutions of collagenase type I and Proteinase K were used to mimic the de- gradation environment. Wistar mice and New Zealand Rabbits were used as subcutaneous and abdominal degradation model *in vivo*. Results showed that it took 8-12 weeks for the tissue to re- pair *in vivo*, while the time intervals needed to get the same degradation percentage for collage- nase I and Proteinase K *in vitro* were 12 h and 60 min respectively. SIS was totally degraded in 24 weeks *in vivo* and the corresponding time for collagenase I and Proteinase K *in vitro* were 96 h and 120 min. Besides, SIS showed good histocompatibility and did not have symptoms of adhesion and hematomas. Results demonstrated the processes of degradation and tissue repair were matched well with each other without immunological rejection and SIS could supply the mechanical strength and biological template during tissue repair, suggesting SIS is a potential surgical bioma- terial for clinical applications.

Keywords

Degradation, *In Vivo* and *In Vitro*, Porcine Small Intestinal Submucosa, Biomaterial

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1.Introduction

Acellular tissue matrix (ACTM) is a natural biodegradable material in which animal-derived tissue undergoes physical and chemical methods to remove immunogenic substances from the tissue and retain the extracellular matrix (ECM). The main function of ACTM material is to provide a place for tissue cell growth, at the same time guide tissue regeneration, and provide a certain mechanical strength for the tissue. The material has good histocompatibility due to the removal of immunogenic substances, and at the same time has a certain mechanical strength to support tissue reconstruction, so it is an ideal tissue repair material. The degradation rate of natural biodegradable materials has a huge impact on the safety and effectiveness of the material implanted in the body. When the material degrades too fast, it cannot provide the biological and mechanical properties of the tissue, which will lead to the failure of the operation and the increase of complications. Degradation is too slow, and it will affect the regeneration and repair of the tissue [1], so the biological material should maintain its own characteristics before the tissue repair is complete, so that the tissue can be rebuilt.

Acellular small intestinal submucosal matrix material (SIS) is a biological material obtained by removing immune components from the small intestine of pig origin through a series of treatments, and retaining ECM. The material is affected by its excellent physical and chemical properties, biocompatibility, and degradable absorption capacity. More and more attention [2], now SIS has been widely used in abdominal wall repair, tendon repair and dural repair. Zhang Xihai and others studied the repair of the abdominal wall defect in the small intestine submucosa, and the results showed that the animals had no adverse reactions, no hernia fistula occurred, and the hernia patch did not rupture after 12 weeks of implantation [3]. Song Zhicheng and others performed a tissue engineering scaffold constructed from the submucosa of the small intestine and tenocytes to repair the abdominal wall defect in rats. The experiment showed that blood vessels grew in and the muscle tissue grew in the junction area between the scaffold and muscle tissue, and the mechanical properties showed that the mechanical strength of the scaffold was greater than that of SD rats. Abdominal wall strength [4]. Although the materials have been proven to have good compatibility and high-strength mechanical properties, there are few reports on the comparative studies on the degradation of materials in vivo and in vitro. In this paper, SIS was selected to carry out in vitro degradation experiments of type I collagenase and proteinase K, as well as animal subcutaneous implantation and abdominal wall repair experiments, to compare the degradation trends in vivo and in vitro, and to explore the correlation between degradation and tissue repair. The research provides theoretical basis [5] [6] [7].

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.; proteinase K

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(Merck, Germany, Merck); type I collagenase (C0130, Sigma). Proteinase K: accurately weigh 20 mg of enzyme, dissolve it with PBS and dilute to 100 mL, take 2 mL of constant volume solution and dilute to 100 mL with PBS solution. Type I collagenase: accurately weigh 50 mg of type I collagenase, dissolve it with PBS, and dilute to a 100 mL volumetric flask.

2.2. Main instruments

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

2.3. Type I collagenase degradation experiment

Take 1 × 2 cm2 SIS, dry, weigh, and put it into a 5 mL centrifuge tube, add type I collagenase (sample: enzyme solution = 4 mg: 1 mL) according to a certain ratio, and react in a constant temperature shaker (37℃, 200 rpm). The samples were taken out at 3 h, 6 h, 9 h, 12 h, 20 h, 28 h, 36 h, 48 h, 60 h, 72 h, and 96 h, and the samples were dried and weighed to calculate the degradation rate.

2.4. Proteinase K degradation experiment

Take 2 × 0.7 cm2 SIS, dry and weigh, add proteinase K according to a certain ratio (sample: enzyme solution = 5 mg: 1 mL), water bath 56 ℃, respectively at 15 min, 30 min, 45 min, 60 min, 75 min , 90 min, 105 min, 120 min Take out the sample and weigh it, calculate the sample product degradation rate.

2.5. Subcutaneous degradation experiment in rats

Sixteen Wistar rats (regular weight 100 g ~ 140 g, obtained the animal ethics approval of the Key Laboratory of Blood Safety and Security of the PLA Institute of Field Transfusion, Academy of Military Medical Sciences of the Chinese People's Liberation Army) were randomly divided into 4 groups: the first week group, The 4th week group, the 8th week group and the 12th week group (4 in each group, half male and half female). Each rat was anesthetized by intraperitoneal injection of 3% sodium pentobarbital (30 mg/kg body weight). Under aseptic conditions, a longitudinal incision was made in the skin in the middle of the abdomen, the subcutaneous tissue was bluntly separated from both sides, 40 mm × 70 mm SIS was embedded, the skin was sutured, and intramuscular injection was performed. Doxycycline (20 mg/kg body weight), once a day for 3 consecutive days, normal feeding, eating and drinking. At different times, the rats were put to death by anesthesia, and the absorption and degradation of SIS at the implanted site were observed visually, and recorded。

2.6. Rabbit Abdominal Wall Implant Degradation Experiment

New Zealand rabbits (regular weight 2.5~3.0 kg, approved by Animal Ethics Institute of Shandong Academy of Medical Sciences) were weighed and anesthetized by intravenous injection of 3% sodium pentobarbital at a dose of 1.5 mL/kg. After the rabbit is anesthetized, lie on its back and fix it, remove the coat on the abdomen, and disinfect with iodophor. Spread a sterile hole towel,

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Table 1. Mainly used instruments in this study

Instrument	Model	Manufacturer
Medical centrifuge	TG16-WS	Xiangnan Xiangyi Laboratory Instrument Development Co., Ltd.
Desktop constant temperature oscillator	BSD-TX270	Shanghai Boxun Industrial Co., Ltd. Medical Equipment Factory
UV-visible spectrophotometer	WF-ZUV-2100	Yonex (Shanghai) Instrument Co., Ltd.
Medical packaging performance tester	MED-1	Jinan Languang Electromechanical Technology Co., Ltd.

Make a longitudinal incision of about 8 cm along the midline of the abdomen, bluntly peel off the skin and subcutaneous tissue, 1 cm from the midline of the abdomen, cut off the abdominal wall tissue of 2 cm × 3 cm, and cut 3.5 cm × 2.5 cm SIS , Sutured to the abdominal opening in the peritoneum with intermittent sutures all around. After observing whether there is bleeding, suture the subcutaneous tissue and skin in turn. The operation picture is shown in Figure 1. Cefoperazone sodium and sulbactam sodium were injected intravenously at the ear to disinfect the surgical site and put it back into the cage. Routine anti-inflammation was performed 5 days after the operation, and the physiology of the rabbits was closely observed. At 2 weeks, 4 weeks, 8 weeks, 12 weeks, and 24 weeks postoperatively, the animals were anesthetized with 3% sodium pentobarbital, and the animals were sacrificed by bleeding from the femoral artery for material degradation assessment.

3. Results

3.1. Type I collagenase degradation

As shown in Figure 2, the in vitro degradation process of type I collagenase shows that the quality of the sample gradually decreases with the extension of the degradation time, and the sample in the degradation solution gradually decreases with the extension of the degradation time. The solid fragments of the experimental sample are 96 h after enzymatic hydrolysis The quality is almost zero. From the degradation rate and the degradation curve, it can be seen that the degradation rate of the sample at 6 h is about 29%, the degradation rate at 12 h is 40%, the degradation rate at 20 h is about 68%, and the degradation rate of the sample at 96 h is over 90%.

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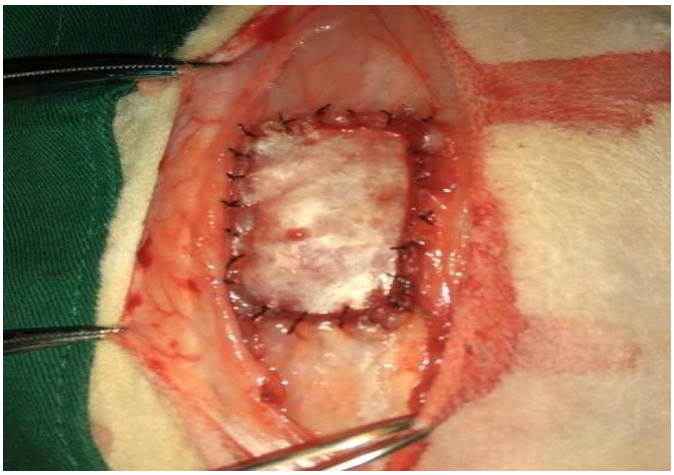

Figure 1. The picture of SIS implanted into abdomen

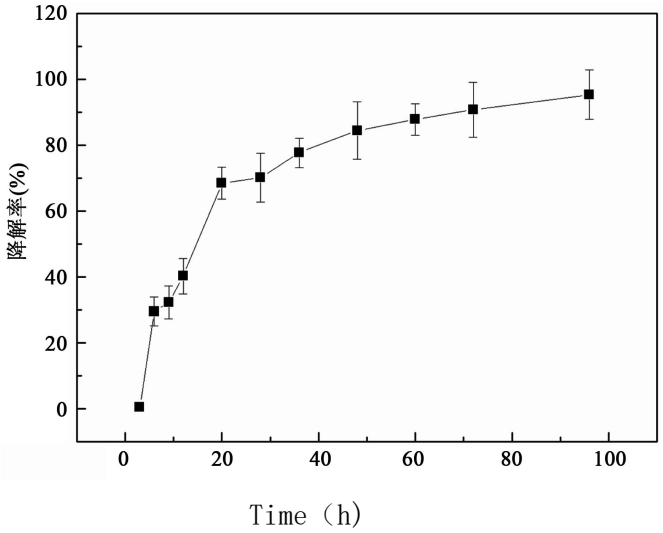

Figure 2. In vitro degradation curve of SIS dissolved in protease solution of type I collagenase

3.2. Proteinase K degradation

Further use of proteinase K for in vitro degradation experiments is shown in Figure 3. Compared with type I collagenase, proteinase K has a stronger enzymatic hydrolysis ability for SIS materials and a faster in vitro degradation process. From the degradation curve, it can be found that the degradation rate is 29.67% at 15 min, more than half of the samples have been degraded at 60 min, and the degradation rate is 56.33%, most of the samples have been degraded at 90 min, and the degradation rate is 71.62% at 120 min more than 90.

3.3. Subcutaneous degradation in rats

The rat subcutaneous implantation experiment was used to observe the degradation of SIS in vivo. After SIS material was implanted subcutaneously in the rat abdomen for 1, 4, 8, and 12 weeks, as the implantation cycle prolonged, SIS was gradually absorbed. One week after implantation, a complete SIS can be seen, and the sample is surrounded by a small amount of connective tissue, which is easily peeled off; 4

DOI: 10.12677/bp.2017.73005

weeks after implantation, the volume of the SIS begins to decrease (it is estimated to be reduced by about one-fifth by visual observation), and the connective tissue around the sample The tissue increases, but it is easy to peel off; 8 weeks after implantation, the SIS volume is significantly reduced (about two-fifths), and the sample is surrounded by a large amount of connective tissue, which can still be peeled off; 12 weeks after implantation, the SIS basically completes tissue repair and reconstruction , The sample (slightly more than one-half retained) is tightly wrapped by connective tissue, and it is not easy to peel off. Based on this, it is estimated that the material degradation rate of SIS after 1, 4, 8, and 12 weeks after implantation in the body is 0%, 20%, 40%, and 45%, respectively, and the degradation curve is shown in Figure 4.

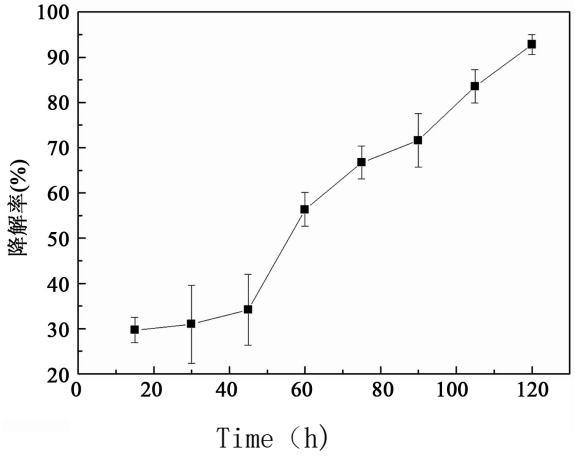

Figure 3. Degradation curve of SIS dissolved in protease K solution

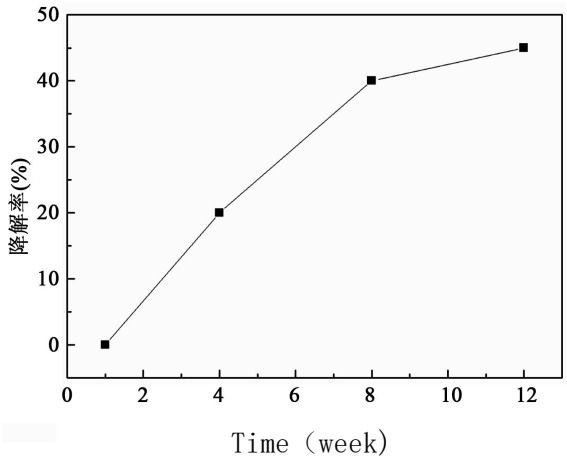

Figure 4. Degradation curve of SIS implanted into rat subcutaneous tissue

3.4. Rabbit abdominal wall implantation experiment

The SIS was implanted into the rabbit's peritoneum, and the degradation process of the sample was observed in the peritoneum. It was found that there was no adhesion between the SIS and surrounding tissues, no prolapse, deformation, or displacement. No blood clots were seen around the patch, and no fibrous capsule formed around the

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patch. The degradation curve is shown in Figure 5. After SIS was implanted into the animal's intraperitoneal wound for 2 weeks, 4 weeks, and 8 weeks, the material was soft and compliant, and no shrinkage was found. The material and tissue were fused at 16 weeks and 24 weeks. Cannot be separated. After 2 weeks of implantation, the intact SIS can be seen with almost no degradation, and the surrounding material is infiltrated by a small amount of fibrous tissue, which is easily separated from the surrounding; 4 weeks after implantation, the SIS is partially replaced by the tissue (it is estimated to be reduced by about a quarter by naked eye observation)), the fibrous tissue around the material increases, but it is easy to peel off; 8 weeks after implantation, the part of the SIS replaced by tissue increases (about one-half), and the surrounding material is infiltrated by a large amount of fibrous tissue, which can still be peeled off; 16 weeks after implantation SIS has basically completed tissue repair and reconstruction, and the surrounding material (about four-fifths) has been completely fused with the newborn peritoneum and cannot be separated; after 24 weeks of implantation, the peritoneal tissue has basically been completely regenerated, and the material cannot be observed, as shown in Figure 6. Show.

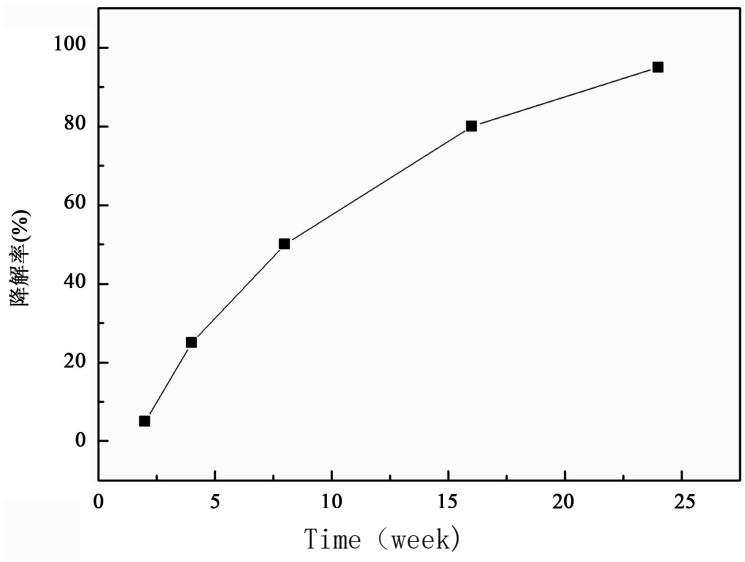

Figure 5. Degradation curve of SIS implanted into abdomen of rabbit

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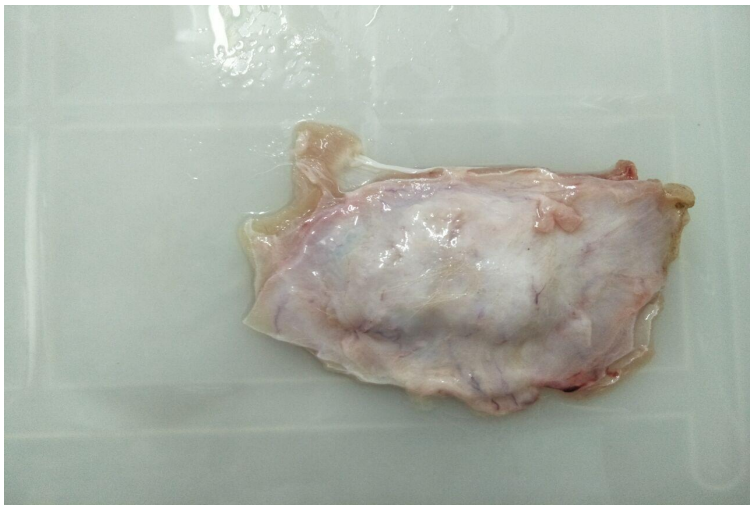

Figure 6. Tissue repaired after implanting SIS into abdomen for 24 weeks

3.5. In vivo and in vitro fitting

The above-mentioned in vivo and in vitro degradation experiment results can indicate that SIS is a degradable material. The material can basically complete tissue repair and reconstruction at 12 weeks. The degradation time of type I collagenase in vitro is about 12 hours. The degradation in vivo in 16 weeks corresponds to degradation in vitro. h, and 24 weeks in vivo degradation corresponds to 96 h in vitro degradation. From the analysis of the amount of material degradation, the critical time point of in vitro degradation time is 12 hours (corresponding to 8-12 weeks in vivo). At this time, most of the material degradation occurs, which means that most of the materials in the body are degraded and absorbed, and the mechanical properties are poor. To complete the purpose of material reinforcement, repair, and reconstruction of defective tissues, the in vitro simulated degradation process of the material should be controlled at a 12-hour degradation rate of less than 50% (type I collagenase), or a 60-minute degradation rate of less than 56.33% (proteinase K). The 4 weeks in the initial stage is equivalent to 6 h (type I collagenase) and 30 min (Protease K) in the in vitro degradation experiment, and the 16 weeks in the later stage of implantation is equivalent to 48 h (type I collagenase), 105 in the in vitro degradation experiment. min (Protease K), the time point of complete degradation in vivo is equivalent to 96 h (Type I collagenase) and 120 min (Protease K) in the in vitro degradation experiment. The degradation data is shown in Table 2. The 12-week in vivo degradation compared to the in vitro degradation time is shown in the dotted box in Figure 7, and the time for complete degradation in vivo corresponding to in vitro degradation is shown in the solid box in Figure 7.

DOI: 10.12677/bp.2017.73005

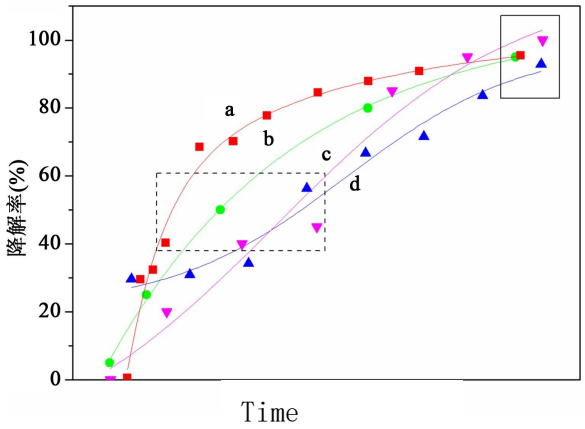

Figure 7. Degradative trend and simulation in vivo and in vitro ((a) The degradation curve of type I collagenase; (b) the degradation curve of abdominal implantation; (c) The degradation curve of subcutaneous implantation; (d) The degradation curve of proteinase K)

Table 2. Comparison of degradation rate and time *in vivo* and *in vitro*

Time (h)	Degradation rate (collagenase) (%)	Time (min)	Degradation rate (Protease K) (%)	Time (w)	Degradation rate (subcutaneous implantation) (%)	Time (w)	Degradation rate (abdominal implantation) (%)
3	0.46 ± 0.21	15	29.67 ± 2.79	1	0	2	5
6	29.46 ± 4.40	30	30.97 ± 8.63	4	20	4	25
9	32.27 ± 4.95	45	34.17 ± 7.80	8	40		
12	40.24 ± 5.42	60	56.33 ± 3.72	12	45	8	50
20	68.54 ± 4.91	75	66.72 ± 3.63				
28	70.19 ± 7.45	90	71.61 ± 5.93			12	70
36	77.76 ± 4.48						
48	84.47 ± 8.72	105	83.57 ± 3.65	16	85	16	80
60	87.86 ± 4.71						
72	90.82 ± 8.36			20	95		
96	95.42 ± 7.53	120	92.80 ± 2.15	24	100	24	95

4. Discuss

With the increase in clinical demand, a series of complications of biomaterials, such as intestinal infarction, intestinal fistula, abdominal wall adhesions, etc., have been found to have a greater relationship with the degradation rate of materials, so it is clear that the law of material degradation It is of great significance for follow-up work [8]. Material degradation includes in vivo degradation and in vitro simulated degradation. In vivo degradation usually involves implanting materials under the skin

DOI: 10.12677/bp.2017.73005

or repairing sites, observing and recording material degradation and tissue reconstruction. This method is closer to clinical application, but it has high costs and long cycles. And there are shortcomings such as individual differences between different animals. In vitro degradation experiments generally involve immersing materials in simulated body fluids (such as PBS, SBF, etc.) or enzyme solutions (such as collagenase, protease, etc.) to observe the degradation of materials. Method Due to the simplified model without the participation of cells, it has the advantages of good controllability and short cycle. As an animal test and preclinical rapid evaluation method, it can reduce the amount of animal use and the cost of animal experiment research in the material development process. The material development cycle plays an important role [5] [6] [7]. Therefore, the establishment of the correlation between the internal and external degradation laws of SIS materials is of great significance for understanding the degradation behavior, tissue compatibility and material development of SIS materials. In this experiment, type I collagenase and proteinase K solution were used to simulate the in vivo degradation experiment, and the rat subcutaneous implantation experiment and rabbit abdomen repair experiment were used to study the degradation behavior of the material in vivo, fit the degradation curve of the material in vivo and in vitro, and establish the correlation between in vivo and in vitro degradation.

SIS material contains type I collagen, a small amount of type III and IV collagen, and also contains a variety of cytokines, such as fibroblast growth factor, transforming growth factor, vascular endothelial growth factor, etc., in addition to elastic fibers, mucin, GAGs and other ingredients [9]. Type I collagenase is type I

The specific hydrolase of collagen, the collagen content in SIS material is as high as 93%, and type I collagen accounts for about 40% of the total collagen. From the in vitro degradation process data of type I collagenase, it can be found that the material gradually becomes smaller and the structure becomes looser with the extension of the degradation time.

The degradation rate is relatively fast at the initial stage. The degradation rate of enzymatic hydrolysis reaches 40% in 12 hours, and the degradation rate reaches about 68% in 20 hours. The content of type collagen gradually decreases, and the degradation rate reaches more than 90% in 96 hours after enzymatic hydrolysis.

The material is completely degraded. This may be because type I collagen, as the main component of SIS, has a certain maintenance effect on the material structure. When it is destroyed, the material loses support and the three-dimensional structure is destroyed. Furthermore, proteinase K was used for in vitro degradation experiments. Compared with type I collagenase, proteinase K is a serine protease with wider cutting activity, which can be used to digest various proteins and is a broad-spectrum protein digestion enzyme. From the experimental data, it can be seen that the digestion speed of proteinase K is significantly higher than that of type I collagenase. This is because in addition to type I collagen, the SIS material also contains other proteins, including type III collagen, type IV collagen, and some elastin. And so on, these can be used as the digestion target of proteinase K. In proteinase K, the degradation rate of the material at 60 min is 56.33%, and the degradation rate at 120

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min is more than 90%.

After implanting the material into the subcutaneous or abdominal wall of the rat, it is found that the degradation process of the material in the two tissues is similar. After the SIS is implanted in the body, as the implantation time increases, the SIS is gradually absorbed and combined with the surrounding tissues, and finally Complete the repair and reconstruction of the organization. By observing the changes in the material in the body, it was found that the material did not change after 1 to 2 weeks of implantation. By 4 weeks, the material began to degrade. By 12 weeks, the material had degraded about 50%, and by 24 weeks, the peritoneal tissue was almost completely. Regeneration, the material cannot be observed, and the material can be considered to have been completely degraded.

Comparing the results of in vivo and in vitro degradation, it can be seen that the in vivo and in vitro degradation curves of SIS materials are similar. From the analysis of the degradation amount, the initial 4 weeks of implantation in vivo is equivalent to the 6 h (type I collagenase) and 30 hours of the in vitro degradation experiment. min (Protease K), 12 weeks after implantation is equivalent to 12 h in vitro degradation (collagenase), or 60 min (Protease K), and 16 weeks in the later stage of implantation is equivalent to 48 h in vitro degradation experiment (collagenase), 105min (Protease K), the time point of complete degradation in vivo is equivalent to 96 h (collagenase) and 120 min (Protease K) in the in vitro degradation experiment. The degradation rate in vitro is faster than the degradation rate in vivo. The possible reason is that the concentration of enzymes in vitro is higher than that in vivo, and the enzymes in the liquid environment in vitro can easily contact the entire body of the material. Sun Jiao et al. studied the biodegradability of polyglycolide-lactide in vivo and in vitro and found that the in vitro degradation rate is lower than the in vivo degradation rate [10]. The degradation mechanism of matrix materials in the body is different. The degradation of artificial synthetic materials is mainly carried out by hydrolysis, while the degradation of extracellular matrix materials is mainly enzymatic degradation [11].

In summary, by studying the degradation behavior of SIS materials in vitro and in vivo, it is found that the degradation behavior in vitro and the degradation behavior in vivo have a certain correlation, and the degradation rate in vitro is faster than the degradation rate in vivo. This study initially reveals the correlation between in vivo and in vitro degradation, provides a reference for in vitro simulation of in vivo degradation, helps to quickly evaluate the degradation behavior of materials in vitro, and shortens the development cycle of biodegradable biopatch.

Thanks

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