Since their first approval by the US Food and Drug Administration, and their introduction into plastic surgery practice, barbed surgical sutures have successfully achieved superior cosmesis, and have been widely used in a variety of experimental surgical procedures, including urology, arthroplasty, orthopedics, obstetrics and gynecology (Lin et al. 2016). The presence of directional barbs fabricated on the surface of monofilament sutures allows free movement of the suture in one direction, while generating resistance in the reverse direction, because the barbs engage with the surrounding tissue. The advantages of using barbed sutures include, but are not limit to, the elimination of knot tying, a uniform distribution of stress along the suture and more efficient wound apposition.

Polydioxanone (PDO) is one of the most popular and commonly used absorbable barbed sutures on the US commercial market. More recently, a new generation of absorbable aliphatic polyesters, poly-4-hydroxybutyrate (P4HB), has received clearance from the US FDA as a novel suture material (Williams et al. 2013). P4HB has good potential for being used as a barbed suture device on account of its higher mechanical strength, prolonged degradation profile and great biocompatibility in living tissue. The primary goal of this study was to discover the feasibility of using P4HB as a potential long-term absorbable material for barbed suture applications. In particular, the approach focused on three different aspects of this question by studying its hydrolytic degradation, in vitro anchoring performance, and in vivo inflammatory response using an animal model.
PDO and P4HB barbed sutures were fabricated in the laboratory using a prototype mechanical barbing machine. Various material characteristics such as the mechanical, thermal and structural properties of both suture materials were tested using different analytical techniques. A 10-week hydrolytic degradation study was conducted to discover the effect of hydrolytic degradation on the changes in mechanical properties and barb morphology of PDO and P4HB barbed and non-barbed sutures. The anchoring performance of a barbed suture, which is the essence of its functionality, was measured in vitro mainly by a suture/tissue pullout test and a wound closure test. The in vivo anchoring performance and the inflammatory response of both types of barbed sutures were studied in a rat model. After the barbing process, P4HB barbed sutures had equivalent tensile properties and anchoring performance to the two commercial barbed suture devices but an inferior anchoring performance compared to PDO. The P4HB monofilaments maintained superior tensile properties compared to the PDO monofilaments over a 10-week hydrolysis period. The in vitro anchoring performance measured as the maximum pullout force was significantly influenced by suture material, suture size, barb geometry, needle type, and tissue type. However, the anchoring performance measured by the wound closure test was related more closely to the suture material rather than the suture size. Due to different suture failure modes, no correlation was found for the anchoring performance measured by these two test methods. The in vivo anchoring performance of PDO barbed sutures decreased continuously over the 28-day implantation period, while the performance of the P4HB barbed sutures increased between Day 14 and Day 28. In general, P4HB barbed sutures generated a less severe inflammatory response than PDO in rat skin tissue. By including images of both the cross-sections and longitudinal-sections, it was possible to directly view the histological interactions between the barbs and the surrounding tissue. Histological barb-tissue interactions were visualized by combining cross-sectional views
and longitudinal tissue sections. In summary, although not typically used as a knotless wound closure device, P4HB shows promise as a barbed suture material, especially for long-term use.
Studies of Barbed Surgical Sutures Associated with Materials, Anchoring Performance and Histology

by

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DEDICATION

To my parents and grandparents.
BIOGRAPHY

Hui Cong was born in Weihai, a coastal city in northern China on September 18, 1989. Taking the advice of her father, who is a textile engineer, she applied to be an undergraduate student at Donghua University, Shanghai, China. She subsequently received a Bachelor of Science degree in Textile Engineering from Donghua University, and a Master of Science degree in Textile Engineering from North Carolina State University, Raleigh, NC, USA. Hui is currently registered as a graduate student in the Doctor of Philosophy degree program with a major in Fiber and Polymer Science and a minor in Biomedical Engineering. Hui has a strong interest in biomedical textiles with a specialty in barbed surgical sutures.
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CHAPTER 1: INTRODUCTION

1.1 Background

A barbed surgical suture is an innovative type of wound closure device that has a plurality of barbs projecting out from the suture main body. Barbs can be fabricated on the surface of conventional monofilament sutures by means of mechanical cutting, laser cutting or by extrusion dies (Ingle et al. 2013). The presence of directional barbs allows free movement of the barbed suture in one direction, while generating resistance in the reverse direction by engaging the barbs with the surrounding tissue. Barbed sutures are also known as knotless sutures. The very first idea of barbed sutures was patented by Dr. Alcamo in 1964 with ten sketches of different barb configurations (Alcamo 1964). The early type of barbed sutures was used for the repair of flexor tendon tissues. However, the results were not successful due to the limitation of the suture material and the barb design (McKenzie 1967). Then in the 1990s, there was a resurgence of interest in barbed sutures with a number of patents published and claims of outstanding cosmesis in plastic surgical procedures (Kress 2008; Villa et al. 2008). So the currently accepted primary clinical application of barbed suture technology is for cosmetic surgery and soft tissue wound closure. Its clinical use in other applications, such as flexor tendon repair, has to date not been approved by the US Food and Drug Administration (FDA). However, experimental studies of using barbed sutures have involved a variety of surgical procedures, such as dermatology, urology, arthroplasty, orthopedics, obstetrics and gynecology (Lin et al. 2016).

Barbed sutures can be classified into two categories based on the barb direction, namely: unidirectional and bidirectional. Unidirectional barbed sutures have a needle attached at one end and a welded anchoring loop at the other end to facilitate fixation. Bidirectional barbed sutures have two needles attached, one on each end, with a non-barbed transition zone in the middle of the
suture length. The deployment of a bidirectional barbed suture starts from the middle of the wound and continues in each direction, while the deployment of a unidirectional barbed suture is the same as for conventional sutures, starting at one end and moving to the other end (Paul & Avelar 2010).

The anchoring performance of barbed sutures is achieved by the engagement of numerous barbs with the surrounding tissue. Therefore, the level of anchoring depends on the frequency and geometry of the barbs, the characteristics of the surrounding tissue, and the interactions between them. The properties of barbed sutures may include, but are not limited to, the type of suture material, suture size, barb geometry, barb alignment and the type of needle (Ruff 2006b). The surrounding tissue may vary from flexible dermal tissue, loose adipose tissue, to compact muscular or tendinous tissue (Ingle & King 2010). The needle-to-suture size ratio and the suturing technique will alter the surrounding force environment that is closely related to the resulting barb-tissue interactions (Shah et al. 2015).

Thanks to advanced developments in polymer synthesis, varieties of polymers are available on the barbed suture market. Among the more desirable suture materials are absorbable polymers with superior tensile properties. They are preferred because it is not feasible to retrieve barbed sutures after their deployment, and there is a high demand to maintain the strength of barbed sutures after the mechanical barbing process. Polydioxanone (PDO) is the most commonly used absorbable barbed suture material on the current commercial market. While PDO was used as a suture material as early as 1981, the first PDO barbed suture was approved for medical use by the United States FDA in 2004 (Ray et al. 1981; Smart & Schaefer 2004). The main degradation mechanism of PDO is hydrolysis, and the hydrolytic mechanism of conventional monofilament sutures has been studied by both in vitro and in vivo methods (Sabino et al. 2000; Ooi & Cameron 2002a; Ooi & Cameron 2002b; Lin et al. 1993; Sevrin et al. 2015). However, researchers have not
yet explored the hydrolytic degradation of PDO barbed sutures. For example, will the degradation rate change with an increased surface area, and will the structural integrity of the barbs change during the degradation process? In addition to PDO, poly-4-hydroxybutyrate (P4HB) is part of a new generation of absorbable polyesters that are synthesized by means of bacterial fermentation (Williams et al. 2013). P4HB has superior mechanical properties with a prolonged degradation profile and is biocompatible with living tissues. P4HB received clearance from the United States FDA in 2007 and is currently used in various medical devices, including monofilament sutures (Martin & Williams 2003). However, little information is available about P4HB barbed sutures.

The tissue anchoring performance of barbed sutures has been measured mainly by two approaches: a suture/tissue pullout test and a wound closure test (Dattilo 2003; Ingle 2004). In the suture/tissue pullout test, a barbed suture is inserted in the tissue in one direction, and then pulled out against the barbs in the opposite direction. The tissue anchoring performance is measured as the maximum pullout force. In the wound closure test, a barbed suture is used to close a wound of specific length and then the repaired wound is pulled apart under tensile loading. The anchoring performance is measured as the maximum wound strength, or alternatively as the force required to generate a 2-mm gap. While these two approaches have been used in different studies, until now no one has investigated whether or not there are correlations between them.

The suture/tissue pullout test and the wound closure test are both mechanical assessments of the extent of a barbed suture’s anchoring ability. In order to directly visualize the interactions between barbs and the surrounding tissues, the present study proposes a histological assessment at the interface between the barbs and the surrounding tissue. Although a number of studies have employed histological techniques to study the inflammatory response and healing process of barbed sutures in living tissue, these histological observations have focused mainly on the inherent
inflammatory response (Kurita et al. 2011; Zaruby et al. 2011; Api et al. 2015). The behavior of the barbs themselves and their interactions with the surrounding tissues were not included. In this study, a combination of longitudinal views and cross-sectional views will be used to observe the histological interactions between the barbs and the surrounding tissue.

In order to determine the answers to the unknown questions mentioned above, a series of studies involving barbed surgical sutures was undertaken involving different materials, and monitoring the anchoring performance and the histological response. Each study is introduced and the results are discussed in the following chapters. In Chapter 3 the focus is on the characterization of material properties for PDO and P4HB monofilaments including mechanical, thermal and structural properties, as well as the fabrication and characterization of barbed PDO and P4HB sutures. Chapter 4 describes the effect of hydrolytic degradation on the change of mechanical properties and barb morphology of PDO and P4HB barbed and non-barbed sutures. Chapter 5 is an account of anchoring performance of these two types of barbed sutures measured by a suture/tissue pullout test and a wound closure test. Chapter 6 reports the findings of in vivo anchoring performance and histological assessment in a rat model. Chapter 7 provides general conclusions and limitations of the experiments described in Chapters 3 to 6, as well as makes recommendations for future work.

1.2 Goal and Objectives

The primary goal of this study was to discover the feasibility of using P4HB as a potential long-term absorbable material for barbed suture applications. In particular, the approach was focused on three different aspects of this question by comparing P4HB with PDO in terms of the
hydrolytic degradation, the \textit{in vitro} anchoring performance and the \textit{in vivo} inflammatory response. In order to achieve this goal, a number of specific objectives are listed below:

I. To characterize the mechanical, thermal and structural properties of PDO and P4HB monofilaments using different analytical techniques such as tensile testing, nanoindentation, scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and X-ray diffraction (XRD).

II. To fabricate PDO and P4HB barbed sutures by using the prototype mechanical barbing machine in the NC State Biomedical Textiles Laboratory, and to compare the barb geometry and mechanical properties of these prototype sutures with two commercially available barbed suture products.

III. To measure any changes in the mechanical properties, thermal properties and surface morphology of PDO and P4HB barbed sutures during a 10-week experimental hydrolytic degradation study.

IV. To discover the effects of suture material, suture size, barb geometry, needle type and tissue type on the \textit{in vitro} anchoring performance of barbed sutures in porcine skin tissue, and to determine the correlation between the anchoring performance measured by a pullout test and a wound closure test.

V. To examine the \textit{in vivo} anchoring performance of PDO and P4HB barbed sutures using a pullout test in a rat model, and to assess the histological interactions between the barbs and the surrounding tissue by making observations in both the cross-sectional and longitudinal directions.
CHAPTER 2: LITERATURE REVIEW

2.1 History of barbed surgical sutures

The concept of barbed surgical sutures was discussed in early 1930s and the first reported trial of using barbed suture to repair a lateral tendon was in 1967 (McKenzie 1967). However, due to a lack of attention and few studies, barbed sutures vanished for nearly half a century. Thanks to the development of new materials and advanced technology, barbed surgical sutures regained focus in the new millennium. Barbed sutures with various configurations and different materials are available in the market and have been presented in numerous clinical cases.

2.1.1 Barbed/Knotless sutures in US patents

The review of patents related to barbed sutures or knotless sutures will be in chronological order. Some designs may look a lot different from the way commercial barbed sutures look on the market now, but they provide the concepts that have moved the evolution of ideas forward.

The original invention of a barbed surgical suture can be traced back to patent USP 3123077A published by John H. Alcamo on March 3, 1964 (Alcamo 1964). Ten different surgical suture configurations shown in Figure 2-1 with raised projections, or depressions, or teeth like barbs or spicules were illustrated to increase the slip resistance of sutures in moist tissue. Alcamo mentioned that these configurations could be achieved by roughening the material surface. However, not all the configurations are manufacturable or even applicable today.
In 1971, Gerald M. Lemole published a patent on a flat suture with latch notches and a latch collar end shown in Figure 2-2 (Lemole 1971). After suturing, the notched suture is pulled through the latch collar and locked, which completes self-latching. This design eliminates the knot tying process which saves substantial surgery time.
In 1978, Taichiro Akiyama patented a suture with spherical projections molded along the suture body at regular intervals as shown in Figure 2-3 (Akiyama 1978). In surgical operations, such as ligating a blood vessel, the hooked needle leads the suture around the blood vessel and then passes through the loop or threading member, which is slightly larger than the projections. After pulling the suture tight, it is securely tied around the blood vessel by the friction among the threading member, the projections, the suture and the blood vessel. According to Akiyama’s claims, the projection can be molded to various configurations such as cone-shaped and bowl-shape.

![Figure 2-3. Knotless suture with spherical projections (Akiyama 1978)](image)

In 1973 and 1981, James C. Tanner Jr. and Wesley W. Walker published patents on barbed sutures, but in short segment form shown in Figure 2-4, which are more like surgical staples. (Tanner Jr. 1973; Walker 1981).

![Figure 2-4. Short segments of barbed sutures (Tanner Jr. 1973; Walker 1981)](image)

Noticing the difficulty of passing a thread through the latch collar or the threading member in Lemole’s and Akiyama’s designs, Peter J. Wilk and David Sekons published a patent on a suture
device which includes a thread, a loop, a connector and a locking component shown in Figure 2-5 (Wilk & Sekons 1992). Similar mechanism as the latch and spherical projection designs, but with the diameter of the loop much larger than the thread, the suture shown below is much easier to use, especially in cases requiring small sutures or ligatures. In addition to closing open vessels, this suture can also be used for tissue approximation without a knot.

In 1993, Inbae Yoon published a knotless suture device particularly useful in endoscopic surgery (Yoon 1993). In this design, the suture morphology may vary from an angled and whisker-like filament to a plurality of tapered barbs shown in Figure 2-6. One common feature of all designs is that there is no needle attached but a sharp distal end which has the same function as a suture needle.
In 1995, Gregory R. Brotz published a patent on barbed sutures shown in Figure 2-7 (a), which is a rigid device and is required to be pushed into tissue (Brotz 1995). In 1996, Brotz published another patent by adding stretchy and elastic connectors shown in Figure 2-7 (b) to the rigid barbed device (Brotz 1996).

![Figure 2-7](image)

Figure 2-7. Rigid barbed suture device (Brotz 1995; Brotz 1996)

In 1994 and 2001 Gregory L. Ruff published two patents on a barbed tissue connector (Figure 2-8(a)) which has conical barbs extending around the periphery of the body (Ruff 1994; Ruff 2001). The tissue connector with enough dimensional stability may not be as flexible as conventional sutures, but has sufficient resilience to integrate with surrounding tissue. The tissue connector can be inserted by hand or by using an inserting device, especially for the one that has multidirectional barbs or the one which is too flexible to be inserted into tissue. This inserting device shown in Figure 2-8 (b) is designed to minimize inserting distortion to tissue and reach different depths of tissue from superficial to a deep wound. Barbs formed by injection molding are yieldable in the inserting direction and rigid in the opposite direction. This ensures a smooth insertion and a strong holding force. The mechanism of barbs eliminates the necessity for tying knots which is highly related to scar formation, tissue necrosis and time-consuming practice.
In 1999, Harry J. Buncke who is known as the father of modern microsurgery published a patent which not only mentions the innovative concept of barbed sutures, but also covers the manufacturing process and ways of deploying both unidirectional and bidirectional barbed sutures (Buncke 1999). In his patent, barbed sutures can be made by physical cutting approaches or laser machine. Buncke introduced various suturing techniques using barbed sutures, especially for facelifts in cosmetic surgery. For the one-way sutures, paired sutures are recommended to maintain anchoring in both directions. The trailing ends of sutures are joined by either thermal bonding (shown in Figure 2-9) or knotting.

Figure 2-9. Two ends of unidirectional barbed sutures joined by a thermal bonding technique (Buncke 1999)
In 2003 Steven D. Morency and Jeffrey S. Jones published a patent on barbed sutures that have a flat suture body with a rectangular cross-section and barbs created on lateral edges shown in Figure 2-10 (Morency, Steven D; Jones 2003). Barbs flex inward to glide through tissue and flex outward to grip the tissue. They showed various barb configurations, such as straight and curved, sharp and rounded, concave and convex curvatures, etc. Barbs could be closely spaced to get higher holding capacity or widely spaced to minimize trauma. Manufacturing processes may include injection molding, stamping, progressive die cutting, and photo-chemical etching.

Figure 2-10. Flat barbed suture with rectangular cross-section (Morency, Steven D; Jones 2003)

In 2004 Leung et al. published a patent that describes barb configurations in detail (Leung et al. 2004) The alignment of barbs on the suture body can be arranged in a staggered disposition, a twist cut multiple spiral disposition, an overlapping disposition, a random disposition or using combinations of the above. In a staggered disposition, different sets of barbs are radially spaced by rotating the suture through a specific angle, such as 180° or 120°. Barbs are offset from the cross-sectional view in order to reduce laceration. In addition to rotating, the suture material can be twisted for 2-17 times, and then be escarped by blade assemblies. After untwisting the suture material, barbs are arranged in a helical pattern. In an overlapping disposition, the cut distance between adjacent barbs is smaller than the barb length. Needles attached to barbed sutures are similar to those for conventional sutures. However, Leung et al. found that the smaller the needle-to-suture diameter ratio, the higher the anchoring performance, which has been tested both in Chamois cloth and rat skin tissue.
In 2007, Nicholas M. Popadiuk et al published a patent mainly describing barbed sutures with an equilateral triangular cross-section as shown in Figure 2-11 (Popadiuk et al. 2007). Two types of barbed sutures were fabricated from polyvinylidene fluoride (PVDF) with similar mass and the same barb configuration. Triangular barbed sutures had 48% strength loss after cutting barbs, while circular barbed suture had 77% strength loss. They demonstrated that barbed sutures with triangular cross-section have superior holding strength compared to those with a circular cross-section by applying a tension and pulling out barbed sutures from a foam block.

Figure 2-11. Barbed suture with triangular cross-section (Popadiuk et al. 2007)

In 2014, Matthew D. Cohen et al. published a patent on a compound barb medical device (Cohen et al. 2014). The innovation of this device is about the barb configuration, more particularly in the inner surface of the barb. The barb inner surface may be composed of one, or two, or three portions, which have different orientations relative to the longitudinal axis of the suture body. Among the three portions, they can be straight, curved or both. Two examples of this design are shown in Figure 2-12.

Figure 2-12. Compound barbs with three straight portions and three curved portions (Cohen et al. 2014)
A number of patents relevant to barbed or knotless sutures have been filed in the US Patent and Trademark Office. Different ideas and designs are listed above, which can portray the evolution of barbed sutures during the past five decades. However, not every idea could be turned into a manufacturable, applicable and profitable product. Designs that have been successfully reduced to practice will be introduced below as commercially available products on the current market.

2.1.2 Barbed sutures in commercial markets

In the market of North America there are three main barbed suture products, Quill™ sutures (Surgical Specialties Inc.), V-Loc™ sutures (Covidien, Medtronic Inc.) and Stratafix™ Spiral sutures (Ethicon Endo-Surgery). The product development, unique features and availability of these three major barbed sutures are described below.

Quill™ bidirectional barbed sutures were launched to the market by Quill Medical Inc. and received clearance from FDA in 2004 (Ruff 2013). At that time, bidirectional barbed sutures were also known as Contour Threads™. In 2006, Quill Medical Inc. was acquired by Angiotech Pharmaceuticals Inc., a global pharmaceutical and medical device company (Anon 2006). In 2013, Surgical Specialties Corporation, a former subsidiary of Angiotech took over the ownership of the Quill™ knotless tissue closure device. According to the statement on the Quill™ homepage, the barbed suture has been used in more than 25 million procedures and presented in more than 80 clinical papers since 2006 (Anon 2015b). According to the product catalog, the bidirectional Quill™ device is available in both absorbable and nonabsorbable materials, including Monoderm™ (polyglycolide(PGA)co-polycaprolactone (PCL)), polydioxanone (PDO), nylon and polypropylene. In addition to bidirectional barbed sutures, unidirectional Quill™ variable loop device is also available. The Quill™ device utilizes a consistent and equal amount of material mass
compared with traditional suture and is available in size 2 down to size 5-0. The size of Quill™ barbed sutures is determined by its outer diameter according to the United States Pharmacopeia (USP). Due to the reduced effective cross-sectional area as a result of creating the barbs, the strength of a Quill™ device is typically rated equivalent to one USP suture size lower than its conventional smooth suture.

In 2009 the V-Loc™ device was launched to the market by Covidien Inc. which was acquired by Medtronic Inc. in 2015. Currently, the V-Loc™ device has two absorbable barbed suture products, V-Loc™ 90 which is made from a copolymer of glycolide, dioxanone and trimethylene carbonate, and V-Loc™ 180 which is made from a random copolymer of glycolic acid and trimethylene carbonate. Permanent or non-absorbable V-Loc™ products are available in polybutester (PBT). It is claimed that the surgeon can save half of the incision closing time by using the V-Loc™ device.

One unique appearance of the V-Loc™ device is the loop at one end of the suture that acts as an initial anchoring point. All V-Loc™ devices have unidirectional barbs on the suture surface. In addition to the direction of barbs, the barb configuration of the V-Loc™ device is also different from that of the Quill™ device. V-Loc™ devices have barbs made by dual-angle cut which is an example of the compound barb mentioned in the previous patent, while Quill™ devices have barbs made by a single-angle cut, in which a broad base tapers to a point. The schematic images and microscopic images are shown in Figures 2-13 and 2-14 respectively. The dual-angle cut is designed to preserve the integrity of the suture strength.
The V-Loc™ device is available from size 4-0 to size 0. However, the size rating system of V-Loc™ is different from the USP system. The caliber of the V-Loc™ device is based on the suture strength, unlike the caliber of Quill™ devices which is based on the core material diameter. “V-Loc™ device 4/0 and Stratafix™* 3/0 have equivalent tensile strengths” according to the V-Loc™ wound closure product catalog, which indicates that 4-0 V-Loc™ barbed sutures are supposed to have similar core diameter to 3-0 Stratafix™ sutures (Anon 2016c). Papers published with measurements of the suture diameter of both Quill™ and V-Loc™ devices demonstrate this difference. Sato et al. measured the diameter of a 4-0 Maxon™ polyglyconate monofilament as 0.209 - 0.239 mm. However, the diameter of 4-0 V-Loc™ polyglyconate barbed suture was 0.274
– 0.304 mm (Sato et al. 2014). Peltz et al. mentioned that the 3-0 V-Loc™ barbed suture has a tensile strength similar to that of a 4-0 conventional suture, which is the same finding as reported by Duffy et al. (Peltz et al. 2014; Duffy et al. 2015). Based on the suture strength caliber system, the V-Loc™ device is typically rated equivalent to two USP suture sizes lower than its conventional smooth suture. When comparing barbed sutures with conventional sutures, it is appropriate to use two sizes larger than the V-Loc™ device, while for Quill™ devices, one size larger would be more appropriate.

Stratafix™ knotless tissue control devices was launched to the market by Ethicon Inc. in 2012 (Anon 2016b). There are two products involved in this new portfolio, the Stratafix™ Spiral device and Stratafix™ Symmetric device. The Stratafix™ Spiral device is similar to the Quill™ device, and available as unidirectional and bidirectional barbed sutures. One difference is that the surface of the unidirectional Stratafix™ Spiral device is applied with antibacterial technology. Stratafix™ Symmetric device is a unidirectional barbed suture, in which barbs are pressed symmetrically on the suture surface in order to maintain the core material. However, Stratafix™ Symmetric device has not yet been launched on the market.

Barbed surgical sutures were available in North America in the early 21st century, while it was launched on the Russian market in the early 1990s (Anon 2016a). Threads with “prominences” are known as Aptos® threads, which stands for anti-ptosis. Aptos® threads are available in polypropylene as a permanent suture and made from the copolymer of PCL and poly-L-lactic acid (PLLA) as a biodegradable suture. Barbed surgical sutures may be known as Woffles Threads in Singapore and by various other trade names, such as Thread Queen TR-Q (Metro Korea Co., Ltd.) and Coco-drilo (Only Medical Co., Ltd.) in South Korea. The Silhouette® suture (Sinclair Pharma, Godalming, UK) is a different type of barbed suture invented by Dr. Nicanor G. Isse (Kress 2008).
Unlike cutting barbs on the suture surface, Dr. Isse put absorbable cones or trumps which are spaced by knots on the suture surface (Figure 2-15). This design is claimed to have more resistance to sagging tissue.

![Silhouette Soft® suture with knots and trumps](Anon 2003)

### 2.2 Mechanical studies of barbed sutures

Barbs created on the suture surface by mechanical cutting reduces the core effective area of the suture, which results in a significant loss in suture strength. In order to maintain the equivalent suture strength, one or two USP sizes greater than the conventional suture size is recommended. Rashid et al. did a mechanical study on breaking strength of 2-0 polypropylene (PP) barbed sutures and 4 various calibers of nonbarbed PP sutures (Rashid et al. 2007). Suture samples were strung on two cylinders with a surgeon’s knot tied in the free ends. Results showed that the breaking strength of the 2-0 barbed suture was in between 2-0 and 3-0 conventional sutures, with no significant difference with the 3-0 conventional suture. The stiffness of the barbed suture was found to be higher than that of the same caliber nonbarbed suture. However, these results may be different with the absence of knots and with suture products from a different manufacturer.

Suture strength is adjustable by choosing different calibers, while the anchoring performance is dependent on suture materials and barb configurations. Ingle et al. created barbs with same barb configuration on six commercially available monofilament sutures, namely,
Biosyn™, Maxon™, Monocryl®, PDS® II, Ethon® and Prolene® (Ingle et al. 2004). The anchoring ability of barbed sutures was measured by pulling the barbed suture out of the tissue simulant (Miracle Towel). The maximum pullout force of these laboratory fabricated sutures was in the range of 1.4 – 2.1 kg, which has been reported to be a high enough force to effectively close wounds in a rat model. Dattilo, Jr. et al. compared the biomechanical performance of PDO barbed suture with conventional PDO suture by measuring the force required to pull sutured skin simulant (Darra chamois) 2 mm apart (Dattilo, Jr et al. 2003). His results showed a significant difference between barbed sutures and knotted sutures, but not between three different configurations of barbs in terms of their cut angle and cut depth as shown in Figure 2-16.

![Figure 2-16. Geometry of a single barb](image)

On the way to learn more about the barb, finite element analysis (FEA) was used to predict the reaction of the barbs to the force generated by the surrounding tissues. Starting with a 2-D analysis, Ingle et al. found that compression occurred at the upper region of the barb while tension happened at the barb inner surface (Ingle & King, 2007). Later a 3-D analysis was conducted along with a point loading experiment to study the mechanical behavior of barbs with different geometries and barb/tissue interactions (Ingle, King, & Zikry, 2010). The results showed that an increased cut angle increased barb flexibility and the tendency of the barb to peel from the cutline, while increased cut depth improved the anchoring ability of the barbs into surrounding tissue.
Hoy and Gingras compared the wound closure stress for barbed sutures and conventional knotted sutures using photoelasticity (Hoy & Gingras 2015). Photoelasticity is a method to determine stress distribution based on the birefringence of the test material. A 4 cm long incision in a tissue foam simulant material was closed by either a Maxon™ monofilament suture or a V-Loc™ 180 barbed suture. A photoelastic coating was applied to the tissue simulant beforehand. Suture-induced photoelastic fringe patterns (Figure 2-17) and fringe numbers were recorded before and during wound flexure which simulated wound opening. The fringe number of both the Maxon™ suture and the V-Loc™ 180 barbed suture fell during flexure of the closed wound simulant, which resulted from the natural stretching of the suture material and subsequent reduction of compressive stress along the incision line. However, the V-Loc™ 180 barbed suture maintained a more constant fringe number distribution than the Maxon™ suture, which indicated that the V-Loc™ 180 barbed suture had more resistance to suture slippage under wound flexure. Additionally, a sharp increase of fringe number was observed at the end of the suture line for both suture devices and for both no-load and loaded conditions. This might be related to the tied knots and the end loop for Maxon™ and V-Loc™, respectively. So even though the knot tying procedure is eliminated for the V-Loc™ barbed sutures, the end loop may still have the same effect as tying knots at the initial anchoring point. This study focused on the difference of stress distribution generated along conventional knotted sutures and barbed sutures using the same continuous running suture technique. The actual suturing technique of a barbed suture, however, may be adapted to avoid the exposure of such large numbers of barbs.
With the help of tissue simulants and computer modeling, the advantages and clinical feasibility of using barbed sutures have been demonstrated. However, both tissue simulants and computer modeling have their own limitations. The structure of the tissue simulant, regardless of whether it is chamois leather or a foam block, is a lot different from the structure and material properties of natural tissue. Computer modeling helps to predict suture/tissue interactions. However, it tends to simplify the surrounding environment. In order to optimize the tissue anchoring performance of barbed sutures in skin and tendon tissues, Ingle et al. fabricated 9 different barb configurations with cut angles ranging from 150°, 160° to 170° and cut depths ranging from 0.07 mm, 0.12 mm to 0.18 mm (Ingle & King, 2010). These needle-less barbed sutures were then inserted into either porcine skin tissue or bovine tendon tissue through a syringe needle. Peak load of the suture/tissue pullout test showed that a flexible barb is more suitable for skin tissue, while a rigid barb is required to penetrate and anchor with tendon tissue. So the future
development of barbed suture technology needs to take into account the physical and biomechanical properties of the surrounding tissue.

Results from various mechanical analyses have characterized the strength and the anchoring ability of barbed sutures. But prior to utilizing barbed sutures in human subjects, animal studies are needed to bridge the gap between the laboratory and the clinic.

2.3 Animal studies of barbed sutures

Deployment of barbed sutures in an animal model is an approach to study the behavior in a living environment and to evaluate the inflammatory response with host tissue. Animal models are chosen as pre-clinical trials because they provide much more information about the safety, efficacy and potential complications.

Jang et al. compared Aptos barbed sutures with conventional monofilament and multifilament sutures using Sprague-Dawley (SD) rats (weight 400g) (Jang et al. 2005). After inserting suture samples in the panniculus carnosus, a 5 cm length of skin was rumpled to a length of 3.5 cm shown in Figure 2-18. The change in length of the rumpled skin as well as the maximum holding strength (MHS) was measured by pulling the suture from the skin tissue immediately after suture deployment, as well as 2 weeks and 4 weeks post-operatively. Undisputedly, the MHS of the barbed sutures was significantly higher than that of the regular monofilament and multifilament sutures. The length of the rumpled skin with the barbed suture gradually increased to 5 cm in two weeks. The thickness of the capsule and myofibroblasts around the sutures were measured by histological sections stained with hematoxylin-eosin stain and a monoclonal α smooth muscle actin antibody, respectively. The results of a thicker capsule and more prominent myofibroblasts indicated that the barbed Aptos threads might generate more scar formation and wound contraction.
Kurita et. al used a Wistar rat model to complete a histological study of barbed sutures, compared to pure gold thread and gold coated barbed sutures over the course of seven months (Kurita et al. 2011). A 20cm long suture segment of each suture type was inserted in the subcutaneous layer. After one, three and seven months of implantation, the sutures and the surrounding subcutaneous tissues were harvested and sectioned for histological analysis. Sections were stained with hematoxylin and eosin (H&E), Elastica van Gieson, or immunostained for α smooth muscle actin (αSMA). The results of H&E staining at one month was in accordance with Jang’s findings. For the observation up to seven months, barbed sutures showed an acute tissue reaction, while the pure gold thread had delayed tissue reactions, and the gold coated barbed suture showed a combination of the previous two. Elastica van Gieson staining showed that collagen gradually replaced cellular components in the capsule over time, and this transition was observed earlier with barbed sutures. The authors concluded that the barbs on the suture surface perform as hooks when under tension, but also induce collagen production in the surrounding tissues.
Jeffrey Zaruby et al. in Covidien Surgical Devices, Inc. conducted an *in vivo* comparison of V-Loc™ 90 device and Quill™ Monoderm device with conventional monofilament Biosyn™ sutures in a porcine model (Zaruby et al. 2011). A total of 192 incisions were made on porcine dorsal skin (shown in Figure 2-19) and randomly closed by one of the three suture devices. Animals were sacrificed for biomechanical testing at Day 0, Day 3 (inflammation), Day 10 (fibroplasia) and Day 21 (maturation and remodeling), and for histological analysis at Days 3, 10 and 21. The intradermal wound holding strength, measured as the maximum load to break the sutured skin tissue, was recorded, together with the mode of suture failure, namely barb slippage, suture breakage or tissue failure. This study was submitted as part of the 510(k) application to the United States FDA for the V-Loc™ 90 device to demonstrate the equivalence of this device with the Quill™ Monoderm product. The barbed sutures were compared with regular monofilament sutures, and there was a lower dehiscence rate and a lower incidence of incisional morbidity in both barbed suture groups. The skin tissue sutured with the V-Loc™ 90 device had an equivalent biomechanical force with that sutured with the Quill™ Monoderm device, even with 1.5 times more barbs. Due to the different barb formation and configuration, V-Loc™ 90 had a primary failure mode of suture breakage, while Quill™ Monoderm failed mostly with barb slippage, which was explained in terms of a greater inflammatory response. Other differences in the results of the two barbed suture groups might be related to the trauma caused by the needle geometry, the needle-to-suture diameter ratio, and the suture material itself, which were not discussed in the article.
Kristen Gingras et al. conducted a comparison between V-Loc™ 180 device and Quill™ PDO following a similar protocol (Gingras et al. 2012). The V-Loc™ 180 device was found to have a significantly higher intradermal wound holding strength at Day 3 and Day 7 and no difference at Days 0, 14 and 28. This was attributed to different device designs, which included differences in suture material, barb configuration, barb density and helical arrangement.

Other than skin closure, barbed sutures have been used in other types of soft tissue wound closure such as in oncology and gynecology which will be summarized below in Chapter 2.4. Studies related to tendon repair using animal models will be summarized with other in vitro flexor tendon repair studies later in Chapter 2.5.
2.4 Barbed surgical sutures in cosmetic surgery and wound closure

2.4.1 Biology of skin tissues

Skin is the largest organ in the human body providing the first layer of protection from the environment. Skin has three main functions: protection, regulation and sensation (Anon 2014). Skin acts as a barrier to protect against mechanical impacts, toxic chemicals, micro-organisms and radiation. Skin regulates body temperature, prevents loss of body fluids and synthesizes vitamin D. Neural networks and receptors in the skin determine the various sensations of heat, cold, touch and pain. In terms of the biological structure of the skin, there are three primary layers as shown in Figure 2-20: epidermis, dermis and hypodermis (Kikelomo & Riaz 2015).

![Figure 2-20. Biological structure of skin tissue (Hoffman 2014)](image)

Epidermis can be then divided into 5 sublayers or strata from the very outer layer: stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale shown in Figure 2-21 (Ash et al. 2013). The keratinocyte is regarded as the principle cell type in the epidermis. Keratinocytes are derived from basal keratinocyte stem cells in the stratum basale,
which is commonly described as a layer one cell thick. Keratinocytes move up to the stratum spinosum which has a spindly appearance to polyhedral keratinocytes which secrete cytokeratin. Keratinocytes migrating up to the stratum granulosum are known as granular cells, which bind intermediate keratin filaments together. During the transition from stratum granulosum to stratum corneum, the cells secrete lamellar bodies, which form the hydrophobic lipid envelope, and at the same time, lose nuclei and organelles. So the flattened keratinocytes in the stratum lucidum are dead and only about 3 to 5 cell layers thick. Stratum lucidum can only be found in thick skin such as on the palms of your hand and the bottom of your feet. Cells in the outermost layer, the stratum corneum, are known as corneocytes. This layer contains 15 to 20 layers of dead cells which regularly slough off to balance the production of new cells. The life cycle of keratinocytes migrating among different strata is called keratinization. Other than keratinocytes, melanocytes, Langerhans cells and Merkel cells are also the main types of cells in the epidermis. The highly cellular and avascular epidermis is the very first barrier against environmental influences.

Figure 2-21. Five strata of epidermis ( Häggström 2010)
The dermis, which is the layer underneath the epidermis, primarily consists of dense irregular connective tissue, and forms the bulk of the skin providing most of the strength (Graham-Brown et al. 2017). The connective tissue includes cells, such as fibroblasts, and large amounts of extracellular matrix that is primarily composed of glycosaminoglycans, collagen fibers, and elastin fibers. The dermis can be divided into two layers: papillary dermis and reticular dermis shown in Figure 2-22(a). Papillary dermis is the upper layer close to the epidermis and composed of fine and loosely arranged collagen fibers (Figure 2-22 (b)). Dermal papillae project toward the epidermis with blood capillaries. On the other hand, the reticular dermis is composed of densely packed collagen, elastin and reticular fibers, which gives the skin its strength, extensibility and elasticity. The dermis is closely compacted with blood vessels, nerves, sensory receptors, and glands.

Figure 2-22. (a) Papillary dermis and reticular dermis in dermis (b) Schematic of tissue components in loose and dense dermis (Alberts et al. 2013)
The hypodermis is the innermost layer of skin, also known as the subcutaneous tissue or superficial fascia. Hypodermis consists primarily of loose connective tissue, mostly collagen, fat tissue, large blood vessels, and nerves (Figure 2-20). The subcutaneous fat in the hypodermis acts as a cushion that protects the inner organs, as a fuel source that provides energy and as an insulator that regulates body temperature (Heather 2016).

**2.4.2 Aging of human skin and cosmetic surgery**

The aging process of skin starts at birth and is a result of two distinct processes: intrinsic aging which is an accumulation of time changes, and extrinsic aging which is more related to environmental influences such as ultraviolet light exposure and smoking (Kikelomo & Riaz 2015). During the intrinsic aging process, skin sags and wrinkles appear due to an increase of tissue atrophy, especially decreases of proteins, blood supply and elastin production.

Aging is a natural process. However, cosmetic surgery can slow down aging or remove aging signs to meet people’s desire for a youthful appearance. Cosmetic surgery is an optional procedure performed on normal body parts utilizing both scientific intervention and artistic creativity (Choi 2015). Common cosmetic surgery include facelifts, eyelid surgery, breast augmentation, rhinoplasty (nose surgery), and abdominoplasty (tummy tucks) (Lee & Sprague 2016). According to the 2014 plastic surgery statistics report, 15.6 million cosmetic procedures were performed in United States alone (Anon 2015a). With respect to cosmetic surgeries with sutures, a suture suspension lift is a common technique used to reposition loose skin and flabby soft tissue to a tighter and younger-looking site. One of the classic suspension techniques is a superficial muscle aponeurotic system (SMAS) lift, which is a tissue tightening procedure relying on the removal of excess skin. A less invasive facial rejuvenation technique is called minimal access cranial suspension (MACS) lift, which involves the use of suture loops to elevate deep
fascial tissue and much less skin excision (Jewell 2016). Innovative barbed surgical sutures play an important role in this “lunch time” facelift (Atiyeh et al. 2010).

2.4.3 Role of barbed sutures in cosmetic surgery: successful cases and complications

Tissue suspension techniques using barbed sutures have drawn the attention of both surgeons and patients, because it achieves uniform tension distribution, is less time consuming, and provides high satisfaction with superior cosmesis. Numerous barbs along the suture axis in a helical pattern are designed to eliminate “cheese wiring” and reduce stress relaxation of soft skin tissue (Paul 2013).

As one of the early inventors, Dr. Gregory Ruff has been involved in many of the distinct processes such as design, characterization and evaluation of barbed sutures. So he masters a comprehensive understanding of new gradients of tension and compression generated by barbed sutures versus conventional sutures (Ruff, 2006). He has used barbed sutures frequently in facial rejuvenation, breast augmentation and tummy tucks, and gained encourage results. Dr. DeLorenzi claimed that 80% of his patients were satisfied with the results of facial rejuvenation (DeLorenzi 2006). Dr. Paul commented that using barbed sutures simplified midface suspension. More surprisingly, it provided the ability to adjust sutures postoperatively (Paul 2006). Later he has used barbed sutures in various open aesthetic fascial procedures, including suspension of the brow and midface, platysmaplasty and lateral neck suspension with considerable success (Paul 2013). Dr. Sulamanidze, who is the inventor of the Aptos® barbed suture has summarized thread lifting procedures done on 4580 patients within 10 years (Sulamanidze & Sulamanidze 2009). They concluded that the advantages of using Aptos techniques were simplicity, minimally invasive, low trauma and sufficient permanence. Dr. Suh and colleagues have reviewed the outcomes of noninvasive thread lifting procedures done with PDO barbed sutures in South Korea from 2012 to
2014 (Suh et al. 2015). They concluded that PDO barbed sutures used in facial rejuvenation are effective with less scarring and a simpler operation setup.

Under the same category but with a different aspect, barbed sutures also perform excellently in wound closure for aesthetic plastic surgery, such as breast and body contouring. Dr. Warner and colleagues have used barbed sutures for progressive tension closure in abdominoplasty which involved 58 female patients between 31 to 57 years old. Results showed that the typical time to complete progressive tension closure has dropped down to 9 minutes with barbed sutures, compared with 15 to 20 minutes with interrupted progressive tension sutures (Warner & Gutowski 2009). Dr. Moya published his experiences on body contouring using Quill™ barbed sutures primarily on abdominal wall, arm, trunk and thigh procedures. He experienced the following benefits of barbed suture technique as: ease of tissue manipulation and wound closure, reduced drain management and improved scar cosmesis, all of which have been mentioned by other authors (Moya 2013).

Using conventional sutures for wound closure, surgeons start the suturing process from the distal end towards him/herself. Using bidirectional barbed sutures, surgeons need to start at the middle point of the wound and proceed simultaneously both proximally and distally to the ends. Even though this is different from the traditional suturing direction, the suturing approach is still the same. Dr. Malcolm D. Paul claimed that completion of 1 to 2 cases with this technology is sufficient to achieve the competency in closure techniques (Paul 2013).

Despite the successful breakthrough of barbed sutures in cosmetic surgery, disappointments and complications have been reported, especially at the initial stage. During a panel discussion about “Thread lift” at the Aesthetic Meeting in 2006, panelists gave a negative impression of barbed sutures. Sixty percent of respondents indicated that barbed sutures were not
a useful practice adjunct, and 59% of respondents thought that barbed sutures created more problems than they solved (Gryskiewicz & Adams 2006). Some complications occurred in Dr. Sulamanidze’s 10-year experience were: hematoma formation, suture extrusion and migration due to an inadequate lifting effect, asymmetric appearance, a visible suture line in superficial skin and one case of allergy (Sulamanidze & Sulamanidze 2009).

The clinical trials of barbed sutures started in cosmetic surgery with the lift of sagging soft tissues. Surgeons and patients have reported both efficacy and safety. Many of the reports were published by surgeons who have had training, practice and positive experiences with barbed sutures, and who have understood how to use barbed sutures to capitalize on their advantages. However, this does not mean that the outcomes of rejuvenation done with barbed sutures will be the same as those that follow more invasive procedures. In order to get an extended lifting time with minimal invasiveness, risk and cost, surgeons need to master the technique of barbed suture insertion instead of just using them as another suture thread.

2.4.4 Barbed suture in other soft tissue wound closure and robotic surgery

In addition to cosmetic surgeries and body contouring operations, barbed suture techniques are also described in wound closure of other soft tissues. The anchoring performance generated by the number of barbs along the suture length secure the wound with an effective and easy running suture technique, such as with a long wound in a joint arthroplasty. The elimination of knot tying also provides benefits in laparoscopic surgery where the suturing space and control of the suture are limited.

An effective and secure wound closure after total joint arthroplasty is imperative. Dr. Ting et al. has performed a clinical trial to evaluate the efficacy of using bidirectional barbed sutures compared with traditional sutures in deep closure of total hip and knee arthroplasties (Ting et al. 2011).
The deep fascia, subcutaneous fat and subdermal layers were closed with barbed sutures, and compared to traditional interrupted sutures. The results showed that it took significantly less suture material and operative time in the barbed suture group compared with the traditional suture group. There were no significant differences in complications, wound cosmesis and patient satisfaction between the two groups. These results are in accordance with Dr. Vakil’s biomechanical in vitro study of cyclic loading on interrupted traditional sutured arthrotomy repair compared with a running barbed suture repair as shown in Figure 2-23 (Vakil et al. 2011). However, in Dr. Campbell et al’s study with 416 patients, they found more complications in knee arthroplasty repair with V-Loc barbed sutures compared with metallic staples (Campbell et al. 2014).

Figure 2-23. Arthrotomy repair with running barbed sutures (Vakil et al. 2011)

An evaluation of the use of the barbed suture technique was compared with traditional sutures for urinary tract reconstruction both in vitro and in vivo using a porcine model (Weld et al. 2006). Equivalent results were found in tissue approximation, laparoscopic pyeloplasty and
bladder neck anastomoses in both groups. Vesicourethral anastomoses repaired with barbed sutures and conventional sutures were compared during robotic radical prostatectomy (Moran et al. 2007). Equivalent results were shown in visual accuracy and security, and the deployment was significantly faster using a barbed suture technique. The author described the combination hybrid technique of barbed suture as “sutureglue”, which appears to overcome two thirds of the theoretical disadvantages. However, clinical trials of urethrovesical anastomosis repaired with barbed sutures in robot-assisted laparoscopic prostatectomy revealed higher cost, more issues with overtightening, delayed healing and longer catheterization compared with conventional sutures (Williams et al. 2010). Dr. Shikanov et al. concluded that a barbed suture was effective, efficient and safe as a conventional technique in an in vivo porcine laparoscopic partial nephrectomy procedure (Shikanov et al. 2009).

Greenber et al. published a review paper of barbed suture technology used in obstetric and gynecologic procedures such as myomectomy, hysterectomy, cuff closure and Cesarean delivery (Greenberg & Goldman 2013). Concluding from 9 different studies, the authors summarized that barbed sutures are, at least, equivalent to conventional smooth sutures for soft tissue approximation with shorter surgical times and less intraoperative blood loss.

Demyttenaere et al. reported the first trial of using a barbed suture technique in gastrointestinal enterotomy closure in 12 pigs (Demyttenaere et al. 2009). Both in vitro and in vivo results showed similar closure efficacy, burst strength, adhesions, histology and inflammation at different time points between the test group (barbed sutures) and the control group (conventional sutures). Recent clinical trials of 50 patients in Singapore also showed the efficacy and safety using barbed sutures in intracorporeal enterotomy closure (Bautista et al. 2016).
Barbed sutures eliminate the necessity of knot tying, which makes the suturing much more efficient in time and space. This benefit is more remarkable in long wound closure and robotic surgery. The anchoring performance of the barbs distributes tension evenly along the wound, which enables improved tissue approximation and rehabilitation. However, barbs exposed on the tissue surface as a running suture, and complications related to barbs, are still considered as clinical concerns.

2.5 Barbed surgical sutures in flexor tendon repair

As mentioned above, the application of barbed surgical sutures in cosmetic surgery and soft tissue wound closure has been evaluated thoroughly. However, its application in tendon repair is still being researched due to the different tissue structure and the higher required mechanical performance.

2.5.1 Flexor tendon and flexor tendon injury

Since barbed surgical sutures have been successfully applied in soft tissues closure, researches and orthopedic surgeons are interested in discovering advantages of barbed sutures in tendon repair. While the biomechanical requirements for tendon repair is much higher than wound closure, so the application of barbed sutures in tendon repair is mainly focused on repairs of small tendons such as digital flexor tendon repair.

The tendon is a tissue connecting muscles to bone. Collagen is the main component of tendon, and accounts for 75% of the tendon’s dry weight, while type I collagen accounts for 97% of the collagen portion (James et al. 2008). Flexor tendons are located on the palm side of the hand. When muscles in the forearm contract, these long flexor tendons pull on the small bones of the fingers and thumb to enable a grip posture. While fingers are bending or straightening, the flexor
tendons are gliding through tendon sheaths shown in Figure 2-24, which keep the tendons in place next to the phalanges.

Figure 2-24. Flexor tendons and tendon sheaths in palm (Jennings & Moseley 2011)

Flexor tendons attached to each finger, from the index finger to the small pinky finger, are divided into the flexor digitorum profundus (FDP) and the flexor digitorum superficialis (FDS) shown in Figure 2-25. While the thumb only attaches to the flexor pollicis longus (FPL).

Figure 2-25. Flexor digitorum profundus and superficialis (Stadnick 2012)

A flexor tendon injury makes it impossible to bend fingers, which brings lots of inconvenience to daily life. According to Jong and colleagues’ 10-year study, there were 33.2 acute
traumatic tendon injuries in the hand and wrist per 100,000 person-years within Olmsted County, MN (Jong et al. 2014). There are three main causes of flexor tendon injuries (Jennings & Moseley 2011). The first one is a deep cut. Because flexor tendons are very close to the surface of the skin, a deep cut, usually work related like construction and food preparation, has a large chance of cutting the flexor tendon into two or more pieces. In addition to work-related injuries, the second cause of flexor tendon injuries is sport-related. Intense sport activities such as football and wrestling can cause laceration of the flexor tendons. “Jersey finger” is a common tendon injury occurred in these sports when one player’s finger is caught and pulled by another player’s jersey. In sports that require a lot of arm and hand strength, such as rock climbing, tendons can also be stretched or torn. This can explain the result found by Jong et al that the 20-29 year old group has the highest incidence of traumatic tendon injuries. The third main cause is health-related, where certain health condition such as rheumatoid arthritis weakens the flexor tendons and makes them more likely to tear.

2.5.2 Flexor tendon repair techniques

Flexor tendon injuries must be treated surgically to bring two broken ends together for healing and rehabilitation. If the treatment is right after the flexor tendon injury, it is called primary repair, which usually results in the best functional outcome. Treatment taken after 3 weeks of injury because of excessive loss of soft tissues is called secondary repair (Griffin et al. 2012). Both primary and secondary repairs rely on surgical sutures to bring two broken tendon ends together.

Figure 2-26 shows two types of sutures used in repairing broken flexor tendons, namely a core suture and a circumferential or peripheral suture. Core sutures are mandatory to connect two broken tendon ends and they provide main strength to the repaired tendon. Core suture repair with locking or grasping loops 10 mm apart from the repair site was recommended to achieve adequate
strength and prevent gap formation (Wu & Tang 2014). The initial trial of the circumferential sutures which are running sutures is to make the repair site tidier with the intention of reducing adhesions. Now it has been found that peripheral sutures can balance the load between core sutures, reduce gap formation and improve repaired tendon strength up to 50% (Merrell et al. 2003).

![Figure 2-26. Core sutures and circumferential suture in flexor tendon repair (Zhao et al. 2004)](image)

During the healing process, it may take up to a year for a repaired tendon to regain its strength and mobility. Repaired tendons exhibit the weakest point at 5 to 10 days after the surgery (Griffin et al. 2012). So maintaining sufficient strength for the repaired tendon during its healing process is very important. Studies have shown that immobilized tendons lose 50% of their initial strength within the first week (Griffin et al. 2012). In order to achieve the function and accelerate the rehabilitation of repaired tendons, protocols involving passive motion and/or active motion are suggested. Schuind et al. reported that forces required for passive mobilization and active mobilization for intact tendons could be expected to be as high as 9N and 35N respectively by using an S-shaped force transducer (Schuind et al. 1992). Even repaired tendons do not break during various rehabilitation protocols, gap formation occurs which may result in adhesion formation and poor functional performance, which may require a secondary repair.
It has been reported that peak gliding resistance of repaired tendons with a 2-mm gap increased significantly compared to repaired tendons with 0 or 1-mm gap, and repaired tendons with equal and greater than 3-mm gap were all caught by the pulley system (Zhao et al. 2004). In general, gaps formed between 2 mm or 3 mm increase the gliding resistance and chances of tendon failure, because they are associated with an impaired range of motion and limited healing, and are considered as a clinical failure ((Jordan et al. 2014). In order to increase the ultimate strength and reduce gap formation, surgeons may increase the number of core sutures traversing two broken tendon ends, or upgrade the suture size, or add peripheral running sutures, or change the suturing technique by involving more locking loops. All the methods mentioned above, however, result in an increased repair site area which compromises the ability of tendons to glide through the tendon sheath and results in adhesion formation generated by excessive fibrosis.

Since the first recorded flexor tendon repair published in 1917, numerous studies have been conducted to improve the ultimate strength, reduce gap formation and adhesion formation (Griffin et al. 2012). However, there is no ideal suture material, nor ideal suturing pattern. A good thing is that the strength of suture materials is not a concern in tendon repair. A bulky repair site and tendon-suture interactions are issues more associated with knots. The number of knots tied, the location of the knots, such as inside or outside the repair site, and the loop patterns, such as the locking loop or the grasping loop, are topics that have been discussed extensively in tendon repair with the aim to improve the tendon-suture interaction and improve the strength of repaired tendons and at the same time reduce adhesion formation.

2.5.3 Role of barbed surgical sutures in flexor tendon repair

The extensive attention of barbed surgical sutures in flexor tendon repair is attributed to the barbs as anchoring points along the suture length. The presence of barbs eliminates the need
for tying knots, which then dispels concerns associated with knot security, bulkiness, and knot location. Interactions between the barbs and the surrounding collagen fibers promote the tendon-suture interactions and distribute the load more evenly. By using barbed sutures, the cross-sectional areas of the repair site is reduced significantly, which allows gliding of repaired tendons within the tendon sheath to occur more smoothly. The use of barbed surgical sutures in flexor tendon repair can be traced back to the 1950s (McKenzie, 1967). However, due to lack of advanced materials, no additional research was published after McKenzie’s preliminary report in 1967 until 2007. In addition to the maximum load to failure and the load to form a 2-mm gap, studies of tendon repair with barbed sutures also focus on the suturing technique, the mode of failure and changes in cross-sectional area. Eighteen studies are summarized chronologically as follow.

As early as the 1960’s, McKenzie discovered advantages of using barbed sutures in flexor tendon repair: less suture material on the tendon surface, less damage and foreign-body reaction, less adhesion formation, and less constriction and distortion of broken tendon stumps, even under tension (McKenzie, 1967). In his study, two types of nylon barbed sutures were mentioned and described in the form of material, suture size, barb size and suturing patterns. Form A involved a uni-directional barbed suture which could be removed, and Form B was a permanent bi-directional barbed suture. Size 3-0 Form B sutures (Figure 2-27) were tested in cadavers compared with G 40 stainless steel wires. The strength of both repairs were about 20-25N with no significant difference. Similar results have also been generated in living canine models. However, the sample size was insufficient to perform statistical analysis and no raw data were recorded.
In 2007, Abathi et al. presented a pilot study on repairing flexor tendons with both barbed nylon sutures (Quill™, size 0 and 2-0) and conventional nylon sutures (Ethibond™, size 2-0 and 3-0) using a Modified Bunnell and Modified Kessler techniques on 160 cadaveric and porcine flexor tendons (Abathi et al. 2007). The mean maximum loads for barbed sutures were 55.9 N and 37.5 N respectively, while for conventional sutures the mean maximum loads were 53.8 N and 31.2 N. However, there is no results of gap formation and the type of failure mode was not recorded.

In 2009, Parikh et al. and Trocchia et al. published their detailed studies on flexor tendon repair with barbed sutures. Parikh et al. repaired 38 cadaveric flexor digitorum profundus tendons with 2-0 Quill™ barbed polypropylene, 4-0 Prolene® monofilament polypropylene, 4-0 Ethibond™ braided polyester and 4-0 FiberWire® composite polyethylene and braided polyester sutures. The suturing techniques for the 2-0 barbed sutures were the authors own knotless three-strand and six-strand repair (Figure 2-28. (a) and (b)). Four-strand cruciate technique (Figure 2-28. (c)) was used with all conventional 4-0 sutures. The mean loads to failure of barbed suture repair were 36 N and 88 N respectively for the two suturing techniques. The mean load to failure for conventional suture repair was 33 N.
The tendon’s cross-sectional size was measured, for the first time, before and after tendon repair so as to measure the repair-site distortion which was calculated as the ratio of cross-sectional area of repaired tendon to that of uninjured tendon. The repair-site distortion of the tendon repaired with barbed sutures was significantly less than all three of the conventional suture repaired tendons (Figure 2-29).

When comparing the failure mode, 93% of the barbed sutures failed due to suture breakage, while 75% of the conventional sutures failed due to knot rupture. The remaining failures were caused by suture pullout. The tensile strength of repairs by barbed sutures is limited by the strength of the suture material, which has a smaller effective area after cutting the barbs. On the other hand the tensile strength of the repairs by conventional sutures is limited by the knot security and tendon-suture interactions.

Trocchia et al. repaired 40 cadaveric flexor tendons with 2-0 Quill™ barbed polypropylene and 3-0 Ethibond™ using a Modified Kessler-Bunnell and a Kessler suturing pattern, respectively (Figure 2-30). The maximum tensile strengths for barbed suture repair and conventional suture repair were 29.6 N and 34.7 N (p = 0.001), while the 2-mm gap formation force was 22.2 N and 22.8 N respectively. In analyzing the failure mode, 100% of the barbed sutures failed due to suture breakage, while 90% of the conventional sutures failed due to knot rupture. The authors mentioned
three factors that govern the strength of barbed suture repair: 1) the number of barbs engaged inside the tendon tissue, 2) the tension distributed on each barb, and 3) the angle at which the material is placed.

Figure 2-30. (a) Knotless Modified Kessler-Bunnell suturing Pattern, (b) Kessler suturing pattern (Trochie et al. 2009)

In 2011, McClellan et al. published a comparative study of tendon repair using three different methods. 66 porcine flexor digitorum profundus tendons were repaired with one of the following techniques: a four-strand knotless technique using size 0 Quill™ barbed polypropylene, a four-strand Savage technique using 3-0 Ethibond™, or a two-strand Kessler technique using 3-0 Ethibond™. The ultimate strength of the repaired tendons using the above three techniques were 72.4 N, 69.2 N and 32.0 N, while the 2-mm gap formation force were 62.8 N, 59.2 N and 23.4 N respectively. C

The change in tendon cross-sectional size with the knotless barbed technique (7.10 mm²) was significantly smaller than for the Kessler technique (14.3 mm²) and the Savage technique (13.6 mm²), even with a larger suture caliber. The number of throws to secure a knot is critical to determine the strength of repaired tendons. Regardless of the location of the knot either within or outside the repaired tendon, it impedes the healing process by either increasing the gliding resistance or compromising the interposition of the tendon halves. Since barbed suture repair relies more on the number of barbs, the longer suture is inserted to ensure enough barbs are engaged, although the quantity has not been well studied. The amount of foreign body material introduced
by a knotted suture with more throws is difficult to compare with that introduced by a barbed suture with a longer length. Marrero-Amadeo et al. published a biomechanical study on comparing 41 cadaveric flexor digitorum profundus tendons repaired by either a four-strand core with three transverse passes using 2-0 Quill™ polydioxanone, or a four-strand core (Tajima and horizontal mattress) plus a running-locking epitendinous suture using 3-0 Surgilon™ and 6-0 Ethilon®. The maximum load to failure of the above two techniques were 50 N and 48 N, while the 2-mm gap loading were 32 N and 42 N, respectively. Both results showed no significant difference. Empirically, they found that the pullout resistance increases with the increased number of transverse passes.

Buschmann et al. published a biomechanical analysis of 30 lacerated rabbit Achilles tendons repaired with a two-strand Kirchmayr technique or a four-strand Becker technique using Quill™ barbed sutures (no description about the size and material), or 4-0 polypropylene conventional sutures (Figure 2-31) (Buschmann et al. 2011). An epitendinous running suture was applied to the Kirchmayr technique with both suture types and the Becker technique with only the conventional suture. The load to failure of repaired tendons was 30 N, 38 N, 23 N and 38 N respectively, which is about one tenth the strength of an uninjured tendon (292 N). In addition, the load to 2mm gap formation was 25 N, 30 N, 14 N and 26 N. The tendon’s post-surgical cross-sectional areas were 9.9 mm², 12.2 mm², 12.7 mm² and 13.5 mm². According to the increased load to failure and reduced enlargement at the repair site, barbed surgical sutures were recommended by Buschmann et al. to be used for human flexor tendon repair.
Zeplin et al. published a comparative study on 60 cadaveric flexor digitorum tendons repaired with either a modified two-strand or a four-strand Kirchmayr-Kessler technique (shown in Figure 2-32) using 3-0 V-Loc™ glycolic-carbonate barbed sutures or 3-0 polydioxanone (PDO) monofilament sutures (Zeplin et al. 2011). The maximum breaking force of the repaired tendons was 38 N, 145.6 N, 94 N and 149.5 N. Significant differences in the maximum breaking force were found with the two-strand Kirchmayr-Kessler technique using different types of sutures. However, Zeplin mentioned that, if an extended suture material could be inserted, it may improve the maximum force which is directly related to the barbed suture material inserted in the tendon tissue.

In 2012, Zeplin et al. published another paper on flexor tendon repair using barbed sutures, but for the first time, in a dynamic model. Based on the result of their last paper, 2 groups of four-
strand Kirchmayr-Kessler technique using either a 3-0 V-Loc™ glycolic-carbonate or a 3-0 PDO were prepared and tested. Another 2 groups using the same suturing technique and suture material but adding an additional peripheral running suture with 5-0 Vicryl™ braided polyglactin were added to the study. For the dynamic cyclic test, the repaired tendons were tensioned for 500 cycles at each of the following loads, 10, 15, 20, 25 and 30 N. Then after cycling, the tendons were pulled to failure. Adding the peripheral suture significantly increased the maximum load. The maximum load of tendons repaired with barbed sutures under cyclic load was increased by 91%. One thing necessary to mention is that the tensile test conducted by Zeplin et al. was on a loop construction, which two tendons were repaired end-to-end. This testing technique doubled the maximum load since the two sides of the same specimen shared the load (Al-Qattan 2011).

In 2013, Lin et al. repaired 22 cadaveric flexor digitorum profundus tendons using a four-strand modified Kirchmayr-Kessler technique either with a size 0 V-Loc™ glycolic-carbonate barbed suture or a 3-0 Ethibond™ braided polyester suture (Lin et al. 2013). The maximum strength of the repaired tendons was 52.3 N and 42.3 N (p=0.01) respectively, while the 2-mm gap formation load was 10.9 N and 11.7 N. Even though the barbed sutures had a significant higher maximum strength and an equivalent gap formation force compared to the conventional sutures, the authors mentioned the difficulties in handling and suturing with the large size barbed sutures. Both the large locking loop and the exposed barbs sitting at the tendon’s surface raised concerns about using a large size V-Loc™ barbed suture.

In 2014, Peltz et al. repaired 30 sheep deep flexor tendons using a novel three-dimensional (3D) four-strand technique (Figure 2-33) with 3-0 V-Loc™ glycolic-carbonate barbed suture and a four-strand Adelaide technique (cross-locked cruciate core repair) with a 3-0 Ticron™ silicone-coated braided polyester suture (Peltz et al. 2014). A dynamic testing profile of 250 cycles from 3
to 30 N was performed before the repaired tendon was stressed in a tensile test to failure. The ultimate load after 250 cycles of repaired tendons was 61.5 N and 48.6 N respectively. According to the relationship between the 2 mm gap formation force and the number of cycles curve, the novel 3D repaired tendon required 102.5 cycles to form a 2-mm gap, while the conventional Adelaide repair required only 24.4 cycles. Peltz mentioned that in order to take advantage of and benefit from barbed sutures, it will be necessary to create a unique suture stitching design instead of merely applying a conventional repair technique. An additional group of 10 sheep tendons were then repaired with the novel 3D technique using conventional braided sutures. They wished to demonstrate that exact tendon apposition could be achieved with equal tension on each strand and improved tendon-suture interaction of the barbed sutures.

Joyce et al. repaired 40 fresh porcine flexor digitorum profundus tendons using the Adelaide technique with a size 2-0 V-Loc™ polybutester barbed suture compared to a size 3-0 Prolene® monofilament suture (Joyce et al. 2014). After static biomechanical testing, the maximum strength of the repaired tendon was 55.5 N and 52 N respectively, while the 2-mm gap force was 46.5 N and 41.5 N (p<0.05). Knotless repair had a significant reduction in the cross-sectional area of the repaired tendon compared to conventional repair. Joyce stated that there were
no difficulties of handling 2-0 barbed suture and the repair time was reduced because there were no knots to tie.

Sato et al. repaired 10 porcine flexor digitorum profundus tendons using a two-strand modified Kirchmayr-Kessler technique with size 4-0 V-Loc™ polyglyconate barbed sutures and with a size 4-0 Maxon™ monofilament suture (Sato et al. 2014). The 2-mm gap formation force of the repaired tendon was 11.8 N and 9.7 N respectively. In determining the 2-mm gap formation point, tendons repaired with barbed sutures were extended to a breaking strain of 6.9 mm, while tendons repaired with conventional sutures failed when the breaking strain was 6.0 mm (p=0.15). The longer range of motion for tendons repaired with barbed sutures may benefit the passive motion during the healing process.

Instead of comparing barbed sutures with conventional sutures, Jordan et al. repaired 40 fresh porcine digitorum profundus tendons using a four-strand modified Kessler (Kirchmayr-Pennington) technique (Figure 2-34) with size 3-0 V-Loc™ polyglyconate barbed sutures and 3-0 Stratafix™ PDO barbed sutures (Jordan et al. 2014). After 15 setting cycles between 5 - 15N, and 250 testing cycles between 5 - 20N, the maximum load for the repaired tendons was found to be 50.7 N and 42.3 N (p<0.05) respectively, while the 2-mm gap formation force was 26.5 N and 24.8 N. Although displacement and stiffness were also recorded, there were no significant differences between these two types of barbed sutures. It is important to note that the V-Loc™ and Stratafix™ use different caliber systems to measure their suture size, so the tensile strengths of the two suture materials themselves will be significantly different. So while we can conclude that the V-Loc™ device has more advantages over the Stratafix™ suture, the two cannot be directly compared.
In 2015, Jordan et al. published another paper comparing the biomechanical properties of porcine flexor tendons repaired with size 3-0 Stratafix™ PDO barbed sutures and size 3-0 Ethicon™ PDS monofilament sutures (Jordan et al. 2015). The two repair techniques, Kessler and Bunnell (Figure 2-35), were developed for conventional suture repair. The Bunnell repair protocol was also used for the Stratafix barbed sutures, and so one of the groups had a peripheral suture, while the other group was without a peripheral suture. Among the four groups, the knotted Bunnell repair gave the highest maximum load, while the knotless Bunnell repair without a peripheral suture had highest resistance to 2-mm gap formation. Compared to the knotted Bunnell repair, the barbed sutures in the knotless Bunnell repair solved the problem of missing anchor points, such as locking loops in the Kessler repair and increased strength via 2-mm gap formation. This also avoids the “purse-string” effect caused by tendon compression, and shortening during knotted Bunnell repair.
Clemente et al. compared the biomechanical properties of 40 porcine flexor tendons repaired with size 2-0 Quill™ polypropylene barbed sutures and 2-0 Quill™ polydioxanone barbed sutures using the new four-strand repair technique shown in Figure 2-36 (Clemente et al. 2015). There was a control group in which the flexor tendons were repaired by a two-strand modified Kessler technique. Since it is not appropriate to compare a four-strand approach with a two-strand repair technique, the result of control group will not be described. The maximum load for the repaired tendons with Quill™ barbed sutures was 50.3 N and 61.5 N respectively, while the 2-mm gap formation load was 38.2 N and 41.0 N. Compared to conventional repair, barbed repair significantly reduced the bunching at the repair site. All the barbed sutures failed due to suture rupture, which indicates that the strength of barbed repair is limited by the nature of the suture material instead of inadequate tendon-suture interaction.

![Figure 2-36. New four-strand knotless repair (Clemente et al. 2015)](image)

Duffy et al. repaired 72 canine superficial digital flexor tendons using a three-loop pulley (3LP) technique and the Bunnell-Mayer (BM) technique (shown in Figure 2-37) with either size 0 monofilament polypropylene sutures or size 0 V-Loc™ barbed sutures (Duffy et al. 2015). The results showed that using the 3LP technique with a polypropylene suture gives the best biomechanical outcome. However, considering the wider suture material that will expose the tendon surface to barbs which may anchor the tendon sheath and generate more undesirable gliding resistance, means that barbed sutures may not always be the most suitable.
Figure 2-37. (a) 3LP repair, (b) knotted BM repair, (c) knotless BM repair (Duffy et al. 2015)

All comparative studies mentioned above are in vitro until Maddox et al. published their in vivo biomechanical analysis of chicken flexor tendon repair. Flexor digitorum profundus tendons of 25 chickens were transected and repaired using either the Parikh et al.’s knotless technique with a size 3-0 Quill™ PDO, or a four-strand Kessler technique with a size 4-0 FiberWire® (Maddox et al. 2015). The chickens were anesthetized 1, 2 or 3 weeks postoperatively, when the repaired tendons were analyzed biomechanically and histologically. Twenty five percent (8 out of 32) of the knotless repairs had a gap that was greater than 2 mm, compared to only 8% (2 out of 24) for knotted repairs. Barbed suture repair showed a higher ultimate strength after the first week postoperatively, with a lower ultimate strength after the second and third weeks. The higher failure rate and reduced ultimate load at Week 2 of the knotless repair may be due to the degradation of the PDO and weakened tendon-suture interaction caused by failed barbs or gelatinases and collagenases generated when cells invading the repair site. There was no difference of inflammation or fibrosis between the two groups, while a vigorous foreign body reaction occurred with the FiberWire® group.
A summary of the 18 studies, in which tendon repair has been attempted with barbed sutures, is listed below in Table 2-1. The application of barbed sutures in cosmetic surgery has already survived three decades, and research using modern barbed sutures for flexor tendon repair is less than 10 years old. All research is at the stage of in vitro studies except for one in vivo study undertaken by Maddox et al using a chicken model. The strength of the repaired tendon largely depends on the tendon-suture interactions, which are directly related to the suturing technique. Suturing techniques for wound closure using conventional sutures may be easily converted to barbed suture. However, this is not applicable to the technique of flexor tendon repair. Most of the authors in the list used a modified method based on the well-known Bunnell and Kessler repair techniques in order to increase the barb engagement with tendons and avoid exposure of the barbs on the tendon surface. Some authors created their own method to achieve the goal of studying the process of innovation with barbed sutures. However, the comparison of barbed sutures and conventional sutures using different repair technologies is not an acceptable experimental design. The common approach of traversing the two broken tendon ends with four strands provides adequate strength to repaired the tendon as well as avoiding an augmented bulky repair site. Both absorbable and nonabsorbable suture materials have been used in these studies. Since most studies are in vitro, the degradation of the suture material is not a concern. Another question in tendon repair with barbed sutures is “What is the appropriate suture size, and which size of conventional suture should it be compared against?” Due to the reduced effective cross-sectional area of the barbed suture, it is accepted that one or two sizes larger than conventional suture allows the two sutures to be equivalent. One should keep in mind that V-Loc™, Quill™ and Stratafix™ barbed sutures have different caliber systems. A tensile test study of all three sutures may give a good reference starting point for each experimental material. Dynamic testing is a good way to represent
in situ loading of repaired tendons, which facilitates the translation of in vitro laboratory results more directly and accurately to the clinic. The average peak load supported by each single strand regardless of suture material and suture size is about 15 N. According to Schuind et al., 35 N is required to actively mobilize the finger. A flexor tendon repaired with a 4-strand barbed suture should be able to support this force. Even the failure modes of barbed sutures are either suture pullout or suture rupture. This does not mean that the barbed suture is not strong enough to repair a broken flexor tendon. Increasing the suture size is the current solution to overcome this problem. Of course, an improvement in the suture strength is more convincing to expand the application of barbed suture in flexor tendon repair without introducing excessive foreign body material and bulkiness at the repair site. In my point of view, the priority now is to design a repair technique or suturing pattern specifically for barbed sutures.
Table 2-1. Summary of flexor tendon repairs using barbed surgical suture technique

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Core repair method</th>
<th>Core strand</th>
<th>Tendon source</th>
<th>Suture material</th>
<th>Suture size</th>
<th>Maximum force (N)</th>
<th>Force on single strand (N)</th>
<th>2mm gap force (N)</th>
<th>Failure-pullout (%)</th>
<th>Failure-rupture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1967</td>
<td>McKenzie Bunnell</td>
<td>2</td>
<td>cadaver &amp; dog</td>
<td>Nylon</td>
<td>3-0</td>
<td>20-25</td>
<td>10-12.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2007</td>
<td>Abathi et al.</td>
<td>modified Kessler/ Bunnell</td>
<td>2</td>
<td>cadaver &amp; porcine</td>
<td>Nylon</td>
<td>0</td>
<td>55.9</td>
<td>27.95</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-0</td>
<td>37.46</td>
<td>18.73</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2009</td>
<td>Parikh et al.</td>
<td>authors own</td>
<td>3/6</td>
<td>cadaver</td>
<td>Quill PP</td>
<td>2-0</td>
<td>36/88</td>
<td>12/14.7</td>
<td>-</td>
<td>7%</td>
<td>93%</td>
</tr>
<tr>
<td>2009</td>
<td>Trocchia et al.</td>
<td>modified Kessler- Bunnell</td>
<td>2</td>
<td>cadaver</td>
<td>Quill PP</td>
<td>2-0</td>
<td>29.6</td>
<td>14.80</td>
<td>22.2</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>2011</td>
<td>McClellan et al.</td>
<td>authors own</td>
<td>4</td>
<td>porcine</td>
<td>Quill PP</td>
<td>0</td>
<td>72.39</td>
<td>18.10</td>
<td>62.84</td>
<td>18%</td>
<td>82%</td>
</tr>
<tr>
<td>2011</td>
<td>Marrero-Amadeo et al.</td>
<td>authors own</td>
<td>4</td>
<td>cadaver</td>
<td>Quill PDO</td>
<td>2-0</td>
<td>50</td>
<td>12.5</td>
<td>32</td>
<td>67%</td>
<td>33%</td>
</tr>
<tr>
<td>2011</td>
<td>Buschmann et al.</td>
<td>Kirchmayr/Becker</td>
<td>2+epi. 3/4</td>
<td>rabbit Achilles</td>
<td>Quill</td>
<td>-</td>
<td>30/38</td>
<td>NA/9.5</td>
<td>25/30</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>2011</td>
<td>Zeplin et al.</td>
<td>modified Kirchmayr- Kessler</td>
<td>2/4</td>
<td>cadaver</td>
<td>V-Loc Glycolic-carbonate</td>
<td>3-0</td>
<td>19/72.8</td>
<td>9.5/18.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2012</td>
<td>Zeplin et al.</td>
<td>modified Kirchmayr- Kessler</td>
<td>4</td>
<td>cadaver</td>
<td>V-Loc Glycolic-carbonate</td>
<td>3-0</td>
<td>72+57</td>
<td>18+14.25</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2013</td>
<td>Toni Lin et al.</td>
<td>modified Kirchmayr- Kessler</td>
<td>4</td>
<td>cadaver</td>
<td>V-Loc Glycolic-carbonate</td>
<td>0</td>
<td>52.3</td>
<td>13.08</td>
<td>10.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2014</td>
<td>Peltz et al.</td>
<td>authors own</td>
<td>4</td>
<td>sheep</td>
<td>V-Loc polyglyconate</td>
<td>3-0</td>
<td>61.5</td>
<td>15.38</td>
<td>after 103 cycles</td>
<td>33%</td>
<td>67%</td>
</tr>
<tr>
<td>Year</td>
<td>Authors</td>
<td>Core repair method</td>
<td>Core strand</td>
<td>Tendon source</td>
<td>Suture material</td>
<td>Suture size</td>
<td>Maximum force (N)</td>
<td>Force on single strand (N)</td>
<td>2mm gap force (N)</td>
<td>Failure-pullout</td>
<td>Failure-rupture</td>
</tr>
<tr>
<td>------</td>
<td>----------------------</td>
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<td>-------------</td>
<td>---------------</td>
<td>------------------------</td>
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<td>------------------</td>
<td>----------------------------</td>
<td>------------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>2014</td>
<td>Joyce <em>et al.</em></td>
<td>modified Adelaide</td>
<td>4</td>
<td>porcine</td>
<td>V-Loc polybutester</td>
<td>2-0</td>
<td>55.5</td>
<td>13.88</td>
<td>46.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2014</td>
<td>Sato <em>et al.</em></td>
<td>modified Kirchmayr-Kessler</td>
<td>2</td>
<td>porcine</td>
<td>V-Loc polyglyconate</td>
<td>4-0</td>
<td>-</td>
<td>-</td>
<td>11.8</td>
<td>62%</td>
<td>38%</td>
</tr>
<tr>
<td>2014</td>
<td>Jordan <em>et al.</em></td>
<td>Kirchmayr-Pennington</td>
<td>4</td>
<td>porcine</td>
<td>Stratafix PDO</td>
<td>3-0</td>
<td>42.3</td>
<td>10.58</td>
<td>24.8</td>
<td>5%</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V-Loc polyglyconate</td>
<td></td>
<td>50.7</td>
<td>12.68</td>
<td>26.5</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>2015</td>
<td>Jordan <em>et al.</em></td>
<td>modified Bunnell</td>
<td>4</td>
<td>porcine</td>
<td>Stratafix PDO</td>
<td>3-0</td>
<td>58</td>
<td>14.5</td>
<td>25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td>82</td>
<td>NA</td>
<td>54</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2014</td>
<td>Clemente <em>et al.</em></td>
<td>authors own</td>
<td>4</td>
<td>porcine</td>
<td>Quill PP</td>
<td>2-0</td>
<td>50.3</td>
<td>12.58</td>
<td>38</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Quill PDO</td>
<td></td>
<td>61.5</td>
<td>15.38</td>
<td>41</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>2015</td>
<td>Duffy <em>et al.</em></td>
<td>3LP</td>
<td>5</td>
<td>dog</td>
<td>V-Loc polybutester</td>
<td>0</td>
<td>57.7</td>
<td>-</td>
<td>-</td>
<td>3%</td>
<td>97%</td>
</tr>
<tr>
<td></td>
<td>Bunnell-Mayer</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2015</td>
<td>Maddox <em>et al.</em></td>
<td>Parikh method</td>
<td>3</td>
<td>chicken</td>
<td>Quill PDO</td>
<td>3-0</td>
<td>12.8</td>
<td>4.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 2-1 Continued**

- Dynamic study
- In vivo study
2.6 New material for barbed sutures in the future

Poly-4-hydroxybutyrate (P4HB) is a new absorbable polymer that is currently being developed for various medical applications (Williams, Rizk, & Martin, 2013). P4HB is a linear polyester with a molecular structure shown as follow in Figure 2-38. As a member of a diverse class of biopolymesters called polyhydroxyalkanoates (PHAs), P4HB is produced by recombinant Escherichia coli K12 fermentation which is different with other polyesters synthetized chemically (Martin & Williams 2003). Following fermentation, the polymer is isolated so as to achieve a high purity that can be converted into monofilaments or multifilament yarns by melting spinning, or into films by compression molding.

![Molecular structure of P4HB](image)

Figure 2-38. Molecular structure of P4HB (Odermatt et al. 2012)

In 2007, P4HB received clearance from the United States FDA to be used as a monofilament suture for soft tissue approximation and/or ligation (Michael & Casey 2015). The unique properties of P4HB as a new medical material include high strength, low tensile and bending modulus, prolonged degradation profile and excellent biocompatibility. The tensile strength of MonoMax® suture made out of P4HB is 35% higher than the PDS II® suture, but with a Young’s modulus only one third that of PDS II® (Odermatt et al. 2012). It has the comparable strength accompanied with the lowest Young’s modulus and bending stiffness when compared with other four commercial products. These mechanical properties provide MonoMax® sutures with improved flexibility, pliability (ease of handling), knot strength, and knot security.
As an absorbable suture, the degradation mechanism is critical as the suture strength decreases dramatically over the time. The primary degradation mechanism is bulk hydrolysis with some level of surface erosion mediated by enzymes. Numerous studies have been undertaken, both in vitro and in vivo, to study the degradation profile (Odermatt et al. 2012). In an in vitro study (Figure 2-39 (a)) of suture degradation in PBS at 37°C, the results show that MonoMax® maintained its initial strength during the first 12 weeks and then lost 20-30% of its strength after 20 weeks. On the contrary, PDS II® showed a steady decrease of tensile strength and no useable strength after 12 weeks. Similar results have been demonstrated in vivo (Figure 2-39 (b)) by implanting suture coils into rabbit’s subcutaneous tissue. MonoMax® retains approximately 50% of its initial strength during Weeks 8 to 12, while PDS II® maintained the same level of strength loss only at Week 4 to 6 and was too fragile to apply a tensile test after 12 weeks. It is reported that a full resorption of P4HB suture takes about 12 to 18 months. This allows absorbable sutures to maintain their strength long enough during the tissue healing process but eventually absorbed after tissue ingrowth.

In addition to the slower and prolonged degradation profile, the hydrolytic product 4HB is another plus for P4HB used as medical materials. 4HB is a natural human metabolite in many organs and, is able to be converted to carbon dioxide and water and eliminated from the body by metabolism (Michael & Casey 2015). Furthermore, 4HB is said to be less acidic than α-hydroxy acid such as glycolic and lactic acids released from polyglycolide (PGA) and poly-L-lactide (PLLA) (Martin & Williams 2003).
Figure 2-39  *In vitro* (a) and *in vivo* (b) ultimate tensile strength of MonoMax® and PDSII®
Biocompatibility testing to measure cytotoxicity, irritation and sensitization, systemic toxicity, interactions with blood and the local effects after sterilization in ethylene oxide, confirm that MonoMax® sutures have excellent biocompatibility and no evidence of toxicity (Odermatt et al. 2012). Histological evaluation of the degradation of MonoMax® suture has been conducted by implanting suture material in rat paravertebral muscles for 64 weeks (Odermatt et al. 2012). The results show a very low tissue inflammatory response and a complete loss of fiber structure after 50 weeks.

There are three companies that prepare P4HB absorbable sutures in the United States (Michael & Casey 2015). Tepha, Inc. is the manufacturing company of P4HB monofilaments, which it fabricates and sells under the tradename TephaFLEX® sutures. Tornier US manufactures the Phantom Fiber® suture which is a braided high tensile suture made from P4HB and used extensively in orthopedic surgery. B. Braun is a German company that packages, sterilizes and sells MonoMax® sutures as mentioned above.
3.1 Suture materials

Two absorbable suture materials, polydioxanone (PDO) and poly-4-hydroxybutyrate (P4HB) were used in the present study. PDO monofilament with a tradename of Monosorb® was obtained from Samyang Corporation in South Korea and P4HB monofilament was obtained from Tepha Incorporation, Lexington, MA, USA. Both materials were delivered in the form of continuous monofilaments on spools shown in Figure 3-1. The PDO monofilament was dyed purple, while the P4HB monofilament was undyed with a clear appearance.

Based on the commercial availability of barbed sutures on the market, and the capability of the mechanical barbing machine in our laboratory, sizes 0, 2-0 and 3-0 were selected for fabrication and evaluation in this study. The diameter of each size of two suture materials is presented in Figure 3-2.
In order to study the characteristics of PDO and P4HB monofilaments, a series of analytical tests, namely, a mechanical tensile test, heart loop test, nanoindentation, differential scanning calorimetry (DSC), X-ray diffraction (XRD), and gel permeation chromatography (GPC) listed in Table 3.1 were performed on size 2-0 monofilaments. The only exception to this was that a mechanical tensile test was performed on all three sizes. The characteristics of these two materials were compared using JMP Pro 13 software (SAS, Cary, NC) with a significance level of 0.05. Differences in tensile properties due to suture material and size were assessed with two-way analysis of variance (ANOVA). Material differences in the bending properties from the heart loop test were examined with Student t-test. Statistical analysis was not performed for the data collected from the nanoindentation, DSC, XRD or GPC tests due to the limited sample size.
Table 3-1. List of analytical tests and corresponding test parameters

<table>
<thead>
<tr>
<th>Analytical tests</th>
<th>Test parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical tensile test</td>
<td>Maximum tensile force, elongation at break, stiffness, work to rupture</td>
</tr>
<tr>
<td>Heart loop test</td>
<td>Bending length, flexural rigidity</td>
</tr>
<tr>
<td>Nanoindentation</td>
<td>Hardness, indentation elastic modulus, creep</td>
</tr>
<tr>
<td>DSC</td>
<td>Melting temperature, crystallinity</td>
</tr>
<tr>
<td>XRD</td>
<td>Interplanar distance, Herman’s orientation factor</td>
</tr>
<tr>
<td>GPC</td>
<td>Polymer molecular weight</td>
</tr>
</tbody>
</table>

3.1.1 Mechanical tensile test

Mechanical tensile testing was performed according to ASTM D2256 Standard Test Method for Tensile Properties of Yarns by the Single-Strand Method. This mechanical tensile test was applied to all three sizes of the two suture materials, which resulted into six groups. The sample size for each group was five. An Instron Model 5544 tensile tester (Canton, MA) equipped with capstan clamps, shown in Figure 3-3, was used to pull the suture material to failure. An initial gauge length of 20 cm and a testing speed of 20 cm/min were used. Load and extension data were recorded and exported by using Bluehill software. The mechanical tensile properties, namely the maximum tensile force, the elongation at break, stiffness and work to rupture of both suture materials, were extracted from the load-displacement curves. The stiffness was measured as the initial linear slope on the load-extension curve. Under these conditions the extension range for PDO and P4HB was 0 – 60 mm and 0 – 40 mm, respectively. The work to rupture was measured as the area under the load-extension curve. The stiffness and the work to rupture were measured using the “Integrate” function in the Origin Laboratory Software.
Typical load-extension curves for the three suture sizes of PDO and P4HB are shown in Figure 3-4. Distinct differences were observed between the two materials and among the three suture sizes. When comparing the same suture size, P4HB invariably had a much higher maximum tensile force than PDO, while PDO had a longer elongation at break than P4HB. Likewise the slope of the load-extension curve for the P4HB monofilaments was always greater than the PDO, even in the plastic region, which indicated that P4HB had greater stiffness.
The results of maximum tensile force (N), elongation at break (mm), stiffness (N/mm) and work to rupture (J) for the three sizes of the two materials are plotted in Figure 3-5. The differences between PDO and P4HB of the same suture size were significant for all four parameters, as well as between the different suture sizes for the same material. The PDO monofilaments had significantly lower values for maximum tensile force, while significantly higher elongations at break compared with the P4HB monofilaments in all three sizes. The P4HB monofilaments had significantly greater stiffness values compared with the PDO monofilaments particularly when measured at the initial slope region. The work to rupture decreased as the suture size was reduced for both types of suture materials. These significant differences in tensile properties had a great impact on the mechanical barbing process and the barb configuration which is discussed later.

After performing the tensile test to failure, the surface morphology of ruptured suture ends was observed by scanning electron microscopy (SEM). In Figure 3-6a, the broken end of a ruptured PDO suture shows a smooth, partly globular appearance associated with the sharp crack propagation on the left. The broken end of the P4HB monofilament shows radial striations propagated from the crack on the right (Figure 3-6b). It is believed that the globular appearance on the ruptured end was formed due to material melting during the stretching process.
Figure 3-5. Tensile properties of suture materials calculated from the load-extension curves, (a) maximum tensile force, (b) elongation at break, (c) stiffness and (d) work to rupture

Figure 3-6. SEM images of ruptured suture ends, (a) PDO and (b) P4HB
3.1.2 Heart loop test

The heart loop test was performed by modifying ASTM D1388 Standard Test Method for Stiffness of Fabrics. A 27 cm length of suture material was formed into a heart-shaped loop, which was clamped with a paper clip. The length of the loop was measured when it was hanging vertically under its own mass (Figure 3-7). Ten specimens were tested for each suture material. The bending length (c) was converted from the loop length (l), using the equation below,

\[ c = l_0 \times f(b) \]

\[ l_0 = 0.1337L, \text{ in which } L \text{ is the unclamped suture length of 25 cm,} \]

\[ f(b) = (\cos q / \tan q)^{1/3}, \text{ in which } q = 32.85 \left(1 - l_0 / l_0\right), \text{ degree.} \]

The flexural rigidity (G) was calculated using the equation below,

\[ G = 1.421 \times 10^{-5} \times W \times c^3 \]

W is suture mass per unit area, mg/cm².

![Figure 3-7. Measurement of loop length, (a) PDO and (b) P4HB](image)

The results of the measured and calculated parameters are listed in Table 3-2 (* p < 0.05 for PDO vs. P4HB). The weight and diameter of the PDO sutures were significantly larger than...
for the P4HB sutures. However, this did not result in any significant differences in the bending length or flexural rigidity between the two suture materials.

Table 3-2. Results of the heart loop test, presented as mean (standard deviation)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PDO</th>
<th>P4HB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unclamped suture length (L): cm</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Loop length (I): cm</td>
<td>4.1 (0.1)</td>
<td>4.0 (0.1)</td>
</tr>
<tr>
<td>Suture weight: mg</td>
<td>27.5 (0.5)</td>
<td>24.5 (0.1) *</td>
</tr>
<tr>
<td>Suture diameter: μm</td>
<td>376 (7)</td>
<td>356 (7) *</td>
</tr>
<tr>
<td>Bending length (c): cm</td>
<td>6.7 (0.3)</td>
<td>7.0 (0.5)</td>
</tr>
<tr>
<td>Flexural rigidity (G): μJoule/m</td>
<td>0.12 (0.01)</td>
<td>0.13 (0.03)</td>
</tr>
</tbody>
</table>

* p < 0.05

3.1.3 Nanoindentation

The nanoindentation test was done using an Ultra Nano Hardness Tester from Anton Paar USA Inc. Ashland, VA. A platform STeP4 equipped with a diamond Berkovich tip was used to indent the materials’ surface. Due to the large length-to-diameter ratio and the curvature of the surface, it was impossible to indent directly on the flat mounted stand-alone monofilaments. Both PDO and P4HB monofilaments were cut into short segments, embedded in glycol methacrylate (GMA) resin and mounted onto aluminum stubs. The resin blocks with the suture materials inside were trimmed to expose a flat surface with a wedge-shaped glass knife. To verify that the direction of sample preparation had no effect on the nanoindentation results, two surfaces were prepared for each suture material in both cross-sectional and longitudinal planes as shown in Figure 3-8 (a - b). Five indentation spots were selected across the diameter with the initial indent made 30 microns
away from the edge of the sample as shown in Figure 3-8 (c - d), and the other four indents spaced approximately 75 microns apart.

As for each indentation, a 3 mN linear force was applied at a rate of 6 mN/min. The indenter was paused for 10 seconds at the peak load and then unloaded at the same rate. The loading and unloading profile for the machine and an example of the corresponding changes in penetration depth for the suture material are shown in Figure 3-9.

![Figure 3-8](image)

Figure 3-8. Indentation spots across the monofilament diameter in (a) cross-sectional sample and (b) longitudinal sample; initial indent on PDO’s (c) cross-sectional surface and (d) longitudinal surface
Three parameters, namely, hardness, indentation elastic modulus and creep were measured by this nanoindentation test. The hardness is calculated by dividing the maximum applied force with the projected contact area. The indentation elastic modulus is calculated as the slope of the unload curve. The creep is the relative change of the indentation depth while the test force is held constant. The results of these three parameters for the two suture materials measured in both the longitudinal and cross-sectional planes are plotted in Figure 3-10. Higher hardness, elastic modulus and percent creep values were found for the PDO monofilaments compared with the P4HB sutures. No changes in these three parameters were observed along the diameter of the P4HB monofilaments, as well as no changes for the hardness of the PDO monofilaments. The differences in indentation modulus and creep between the cross-sectional and longitudinal sections of PDO may be attributed to the roughness of the exposed suture surfaces. In the case of the P4HB sutures, no significant differences were found in indentation modulus and creep measured in the cross-sectional and longitudinal planes.
Figure 3-10. (a) Hardness, (b) indentation modulus and (c) creep of PDO and P4HB (C: cross-sectional, L: longitudinal planes)

3.1.4 Differential scanning calorimetry (DSC)

The thermal properties of the two suture materials were measured by a Perkin-Elmer DSC apparatus under a nitrogen atmosphere. Samples were cut into short segments, between 3 to 5 mg were placed in aluminum pans and sealed. The heating profile was set from 25 °C to a temperature 20 ~ 30 degrees higher than the melt temperature at a heating rate of 10 °C/min. The temperature at the largest endothermic peak was recorded as the melting temperature (T_m). The apparent heat
of fusion ($\Delta H$) was measured as the area under the melt endotherm peaks. The crystallinity ($X_c$) of the material was calculated as the ratio of the measured heat of fusion of the sample compared to that of 100% crystalline material.

$$X_c = \frac{\Delta H}{\Delta H^0},$$

where $\Delta H^0$ of PDO is 140.8 J/g according to Sabino’s study (Sabino et al. 2004).

The DSC thermograms of PDO and P4HB are shown in Figure 3-11. The thermograms for the two materials each had two peaks, which indicates that both crystalline and amorphous material were formed during the melting spinning and drawing processes. The distance between the two peaks, which indicates the relative amount of energy required to form crystals, was larger for the P4HB monofilaments than for the PDO monofilaments. The melting temperature of PDO is 99.8 °C, which is significantly higher than that of P4HB at 60.7 °C. The heat of fusion of PDO is 81.5 J/g, which corresponds to 58% crystallinity. The heat of fusion of P4HB is 63.5 J/g. However, the percent crystallinity of the P4HB sample was not calculated, because the heat of fusion of 100% crystalline P4HB is not known.

![Figure 3-11. DSC thermograms of PDO and P4HB](image_url)
3.1.5 X-ray diffraction (XRD)

The XRD analysis was performed by using a Bruker AXS General Area Detector Diffraction system with a Hi-Star area detector shown in Figure 3-12. A copper source was used for the production of x-rays that had a wavelength of 1.54 Å. The monofilaments were cut into segments and mounted parallel on the sample holder using double-sided tape. Each monofilament segment was aligned right next to each other with no overlap or gap (Figure 3-13). Diffraction data were collected within a 10-minute x-ray exposure for each sample.

![Figure 3-12. XRD apparatus setup](image)

The signals received in the area of the detector are shown as x-ray diffraction patterns in Figure 3-14. The halo in the diffraction pattern indicates an amorphous region in the semi-
crystalline structure, while the high-intensity bright spots indicate crystalline region. The intensity of 2-theta ($2\theta$) and chi ($\chi$), shown in Figure 3-15, were integrated from the diffraction pattern using software called SAXI.

Figure 3-14. X-ray diffraction patterns of (a) PDO and (b) P4HB

Figure 3-15. X-ray diffraction data for PDO and P4HB, (a) 2-theta and (b) chi
The interplanar space, d was calculated using Bragg’s Law,

\[ 2d\sin \theta = n\lambda, \]

where \( n = 1, \lambda = 1.54 \text{ Å} \).

The d spacing for PDO and P4HB was found to be 4.15 Å and 4.05 Å respectively.

The Herman’s orientation factor was calculated using Herman’s Orientation Function,

\[ f_\theta = \frac{1}{2} (3 < \cos^2 \phi > -1), \]

where \( \cos^2 \phi = \cos^2 \chi \),

\[ < \cos^2 \chi > = \frac{\int_{\pi/2}^{\pi/2} I(\chi)\sin \chi \cos^2 \chi d\chi}{\int_{\pi/2}^{\pi/2} I(\chi)\sin \chi d\chi} \]

The Herman’s orientation factor \( f_\theta \) is in the range from 0 to 1. When \( f_\theta = 1 \), the fiber is perfected oriented. When \( f_\theta = 0 \), the crystalline structures are randomly aligned. The Herman’s orientation factor \( f_\theta \) for PDO and P4HB was found to be 0.845 and 0.816 respectively, which indicates that the crystalline structures in PDO monofilaments were more oriented than those in the P4HB monofilaments.

### 3.1.6 Gel permeation chromatography (GPC)

GPC was performed to measure the molecular weight and polydispersity index (PDI) using size exclusion chromatography that separates analytes according to their size. The smaller analytes would be trapped in the porous beads with a longer retention time in the column and therefore would be eluted last. An Alliance Waters HPLC 2695 equipped with a Waters 2414 refractive index detector (Milford, MA) was used to measure the molecular weight. P4HB segments were dissolved in tetrahydrofuran (THF) at 40°C on a hot plate for 15 minutes with continuous shaking. The solution was diluted in THF to a final concentration of 1 mg/mL for delivery to the THF column. PDI is a measurement of the distribution of molecular mass calculated by the ratio of the
weight average molecular weight (M_w) and the number average molecular weight (M_n). The GPC test was not performed on the PDO samples due to the difficulties of dissolving the polymer in THF. Changes in the molecular weight of PDO were obtained from a previous study (Sevrin et al. 2015). The molecular weight of P4HB was measured as 315 kDa, while that of PDO was reported as 134 kDa.

3.1.7 Discussion

Differences in tensile, bending, indentation, thermal and molecular properties of PDO and P4HB monofilaments may be related to the initial polymerization process. PDO is obtained by ring-opening polymerization of the monomer p-dioxanone, while P4HB is produced by means of a recombinant fermentation process. Different machine settings during the melt spinning and drawing processes also introduced dissimilarities between these two materials. The results of the mechanical tensile test showed that P4HB had a significant higher maximum tensile load compared with PDO, which matched with the results published in the literature (Odermatt et al. 2012). The higher tensile load of P4HB may be attributed to the higher molecular weight. However, the result that P4HB had a significantly higher stiffness than PDO was different from Odermatt et al.’s finding due to the different types of P4HB monofilaments used. Three types of P4HB monofilaments are manufactured by Tepha Inc. The P4HB monofilaments used in the present study were received from Tepha Inc. as Tepha Flex, which have the highest strength and modulus, while the P4HB monofilaments manufactured for MonoMax sutures have the lowest strength and modulus. The high molecular weight of P4HB may impede the crystallization of polymer chains and the orientation of the crystalline structure in the monofilament. Therefore, P4HB showed lower values for hardness, melting temperature and Herman’s orientation factor compared with PDO.
3.2 Fabrication of barbed sutures

3.2.1 Mechanical barbing machine

Barbed suture samples were fabricated using a mechanical barbing machine (Figure 3-16) donated by the Quill Medical Company (Genova et al. 2005). Smooth suture monofilaments were placed in the V-shaped groove of the cutting bed and secured with retention knobs. A tamp/pressure plate was used to ensure that the monofilament sat uniformly at the right depth along the length of the groove. Two knobs in the front face of the cutting bed were tightened to stabilize the monofilament. A cutting template was applied on top of the monofilament as a guidance for the movement of the cutting blade assembly. Barbs were formed on the monofilament surface by sliding the blades against the material protruded above the cutting bed. Different lengths of barbed sections were achieved by using different cutting templates and extension devices (not shown in the figure). No significant difference of bending properties was found between two materials, which may indicate that the bending properties depend more on the structure rather than mater.

Figure 3-16. Mechanical barbing machine showing the components
In addition to the PDO and P4HB barbed sutures being manually fabricated in the laboratory, two commercial products, size 2-0 Quill™ PDO and size 2-0 Monoderm™, were included as reference controls. These two commercial products were manufactured with the same mechanical cutting principle but using a computer-controlled automatic barbing machine. All barbed sutures were observed under a Nikon Labophot-polarizing microscope. The optical microscopy images of size 2-0 barbed sutures are shown in Figure 3-17. The rotational position of the barbs had a 120° staggered spacing, which was generated by three cuts with a 120° rotation between each cut. The barb density of homemade barbed suture was 7.7 barb/cm.

![Image of barbed sutures](image)

Figure 3-17. Optical microscopy images of size 2-0 barbed sutures (a) PDO, (b) P4HB, (c) Quill™ PDO and (d) Monoderm™

### 3.2.2 Barb geometry

The geometry of a single barb shown in Figure 3-18 is determined by the cut angle, cut depth and barb length, which follow the Pythagorean theorem:

\[ Dc = L \times \sin(\pi - \theta) = L \times \sin(\theta) \]

The suture diameter, cut depth and cut angle were measured on the microscopic images using IS Capture software (Tucsen Photonics, Fuzhou, China). In order to eliminate the size factor, the cut depth was calculated in percentage using the equation below,
Cut depth (%) = \( \frac{D_c}{D} \times 100\% \)

A target barb geometry was set as 165° cut angle and 30% cut depth.

![Diagram of barb geometry](image)

Figure 3-18. Geometry of a single barb

In addition to the PDO and P4HB barbed sutures manually fabricated in three sizes, two commercial products, size 2-0 Quill™ PDO (Surgical Specialties, PA) and size 2-0 Monoderm™ (Angiotech, PA), were included as control samples. These two commercial products were manufactured using the same mechanical cutting principle but using a computer-controlled automatic barbing machine. The effect of suture material and suture size on the barb geometry was detected by a two-way ANOVA in JMP Pro 13. Specific pairwise comparisons were made using least square means and Tukey-Kramer HSD posthoc analysis. Differences were considered significant at \( p < 0.05 \).

With the same barbing machine setup, the results of the barb geometry in terms of cut angle, cut depth and barb length of the two homemade barbed sutures are plotted along with the two commercial products in Figure 3-19. The average cut angle was in the range of 162.6° to 167.5°, with a significantly higher cut angle for P4HB barbed sutures compared with PDO barbed sutures. No significant differences were observed between the different suture sizes. P4HB barbed sutures had a similar cut depth to Quill™ PDO barbed sutures, which was significantly higher than the cut depth of PDO barbed sutures. Monoderm™ barbed sutures had the lowest cut depth at 15.8%, which resulted in flimsy, less effective barbs. The PDO, P4HB and Quill™ PDO barbed
sutures had a similar barb length, which was significantly longer than that of the Monoderm™ barbed sutures. No significant differences were found among the different suture sizes.

Figure 3-19. Barb geometry of PDO, P4HB, Quill™ PDO and Monoderm™ barbed sutures (a) cut angle, (b) cut depth and (c) barb length

Different lengths of PDO and P4HB barbed sutures were fabricated for three tests, namely, a mechanical tensile test, a wound closure test and a suture/tissue pullout test. The length of the non-barbed section, the barbed section, the transitional section in between and the barb direction
are shown in Figure 3-20. A total length of 55 cm was required to mount the suture specimen on the tensile tester equipped with capstan clamps. In the wound closure test, bidirectional barbs were engaged with the surrounding tissue, and in order to minimize the gap formation in the transitional zone, the central non-barbed length was shortened to 0.5 cm to match the distance between adjacent blades. In the pullout test, only one direction of barbs would be engaged with the surrounding tissue. One bidirectional barbed suture was cut into two halves so as to prepare two unidirectional barbed suture specimens.

Figure 3-20. (a) Barbed suture for mechanical tensile test, (b) barbed suture for wound closure test and (c) barbed suture for pullout test (unit: cm)
3.2.3 Effective cross-sectional area of the barbed suture

It is recognized that the barbing process reduces the effective cross-sectional area of the barbed suture. Ingle et al. presented the effective cross-sectional area of a barbed suture shown as the shaded area in Figure 3-21 (a) (Ingle & King 2010). R is the radius of the suture cross-section. A cut depth of 25% was arbitrarily chosen to simplify the drawing and calculation. However, this purpose only applies to barbed sutures with a single barb or barbs in a linear sequential alignment. For barbs with helical arrangement, the load is not able to undulate along the suture length as in an isotropic matrix. Because the support of the load is terminated at the barb in an anisotropic monofilament where the polymer chains align along the longitudinal direction. For example, the effective cross-sectional area of a barbed suture with barbs staggered 120° apart is presented in Figure 3-21 (b). The effective area left after the barbing process is calculated as 90.5% and 41.4% for option (a) and (b), respectively.

Figure 3-21. Two options with a 25% cut depth for calculating the remaining effective cross-sectional areas of barbed sutures
The effective cross-sectional areas left after cutting barbs on sutures with 120° staggered spacing to different cut depths are shown in Figure 3-22. When the cut depth is smaller than 25% of the suture diameter, the effective cross-sectional area left is calculated by subtracting the white area from the circular area. If the cut depth is x, the effective area left \( f(x) \) is

\[
f(x) = \pi R^2 - 3\arccos(1 - x)R^2 + 3\sqrt{1 - (1 - x)^2(1 - x)}R^2,
\]

where \( R \) is the radius of the original circular suture cross-section.

When the cut depth is equal to or larger than 25% of the suture diameter, the effective cross-sectional area left is an equilateral triangle. If the cut depth is x, the effective area left \( f(x) \) is given by:

\[
f(x) = 3\sqrt{3}(1 - x)^2R^2
\]

![Figure 3-22](image)

Figure 3-22. Effective cross-sectional areas left after cutting barbs on sutures with 120° staggered spacing to different cut depths, (a) cut depth < 25%, (b) cut depth = 25%, and (c) cut depth > 25%

The change in effective cross-sectional area left as a function of the cut depth is plotted in Figure 3-23 according to the two functions listed above. The cut depth and percentage of effective area left for size 2-0 PDO and P4HB barbed sutures are highlighted in the plot. In this study, the
P4HB barbed sutures had a cut depth about 5% deeper than that of the PDO barbed sutures. From Figure 3-23 one can see the effective cross-sectional area left behind on the P4HB barbed sutures was only about half that of the PDO barbed sutures. The purpose of calculating the effective cross-sectional area is a way to explain and compare the reduction of suture strength after different barbing procedures. Option (b) in Figure 3-21 is the smallest possible cross-sectional area that can remain after cutting barbs at three staggered angles 120° apart.

![Figure 3-23. Relationship between the effective cross-sectional area left behind and the cut depth of barbed sutures](image)

3.2.4 Tensile properties of barbed sutures

The results of the tensile properties of both the barbed and non-barbed sutures are plotted in Figure 3-24. Due to the reduced effective cross-sectional area, there was a significant drop in maximum tensile force, elongation at break and work to rupture for the barbed sutures compared to the non-barbed ones. The PDO barbed sutures maintained 58% of their initial maximum tensile force, while the P4HB barbed sutures maintained only 33% of their initial maximum tensile force. Due to the significantly higher initial maximum tensile force of the P4HB monofilaments, the P4HB barbed sutures were stronger than the PDO barbed sutures, even after the higher percentage
loss of maximum tensile force. Comparing the maximum tensile force of the size 2-0 barbed sutures, the P4HB barbed sutures had equivalent maximum tensile force compared with the two commercial products, while that for the PDO barbed sutures were significantly weaker. After the barbing process, both PDO and P4HB sutures lost about half of their initial elongation at break. The changes in work to rupture for the barbed sutures followed similar trends to the changes described above for the maximum tensile force. While change to stiffness before and after barbing were the least significant among these four tensile properties.

Figure 3-24. Tensile properties of barbed and non-barbed sutures (a) maximum tensile force, (b) elongation at break, (c) stiffness and (d) work to rupture
3.3 Chapter Summary

In this chapter, we describe how three different sizes of PDO and P4HB monofilaments were obtained as raw materials for the fabrication of barbed surgical sutures. Before the barbing process, a series of analytical tests were performed to study the “as received” characteristics of these two materials. In the mechanical tensile test, the P4HB monofilaments had a significantly higher maximum force, significantly higher stiffness and significantly lower elongation at break compared to the PDO monofilaments. Similar bending properties were found between the two materials in the heart loop test. In the nanoindentation test, PDO was found to have greater hardness, indentation modulus and creep compared with the P4HB samples. No significant changes in these three parameters were found across the diameter of the P4HB monofilaments. Using DSC, a significantly higher melting temperature and heat of fusion were found for the PDO monofilaments compared to P4HB. The x-ray diffraction results showed that PDO and P4HB monofilaments had similar interplanar d spacing, but that PDO had superior polymer chain orientation.

Various lengths of barbed sutures were fabricated on a mechanical barbing machine. The barb geometry and tensile properties of PDO and P4HB barbed sutures were compared with two commercial products, the Quill™ PDO and the Monoderm™ barbed sutures. Having a lower hardness and less resistance to the cutting blades, P4HB barbed sutures had a significantly higher cut angle, greater cut depth and barb length compared to the PDO barbed sutures. No significant differences in barb geometry were found between the P4HB barbed sutures and Quill™ PDO barbed sutures. The appearance of the barbs on the PDO and P4HB barbed sutures looked similar to the Quill™ PDO barbed sutures. Due to the smallest cut depth for the Monoderm™ barbed sutures, the flimsy barbs were barely noticeable on the suture surface. As a result of the barbing
process, P4HB barbed sutures lost 64-70% of the initial tensile strength of the non-barbed monofilaments. However, the remaining tensile strength was still significantly higher than that of the PDO barbed sutures, and was equivalent to the two commercial products. A comparison in the performance of these two materials, especially between the barbed sutures, will be discussed further in later chapters.
CHAPTER 4: COMPARISON OF POLYDIOXANONE AND
POLYHYDROXYALKANOATE BARBED AND NON-BARBED SURGICAL SUTURES:
THE EFFECT OF HYDROLYTIC DEGRADATION ON MECHANICAL AND
MORPHOLOGICAL PROPERTIES

4.1 Introduction

The first commercially available barbed suture obtained clearance from the US FDA in 2004. Up until now, barbed sutures have been used clinically in various fields, such as cosmetic, plastic, urological, gynecological and orthopedic surgeries (Lin et al. 2016). As polydioxanone (PDO) first introduced as a monofilament suture material by Ethicon LLC in 1983, it has become a well-known degradable polyester used in soft tissue repair and one of the most commonly used absorbable barbed suture materials in cosmetic surgery (Taylor & Shalaby 2013). As an absorbable suture material, PDO retains 50% of its initial strength after 4 to 6 weeks. This absorption rate is sufficient for most soft tissue closure surgeries. However, for plastic surgery, a longer lasting absorbable barbed suture material is preferred to increase the longevity of the cosmesis. A recently developed biopolymer, poly-4-hydroxybutyrate (P4HB) manufactured by Tepha. Inc. (Lexington, MA) has been converted to various resorbable medical devices and received clearance from the US Food and Drug Administration (FDA) for use as a suture material in 2007 (Williams et al. 2013). P4HB has now become more attractive in tissue repair and tissue regeneration due to its great tensile properties, excellent ductility and prolonged degradation profile. However, only one P4HB barbed suture product appears to be currently commercially available. It is known as Bio Thread Lift™ (Buenos Aires, Argentina) (Williams, Rizik, and Martin 2013). Little information and data have been published about this P4HB barbed suture, except for a talk given by Dr. Nora Pertralli in 2012.
PDO and P4HB are both resorbable thermoplastic polymers, and their chemical structures are shown in Figure 4-1. Each vertex represents a carbon atom and attached hydrogen atom(s). Both PDO and P4HB have three methylene groups (-CH$_2$-) and an ester group (-CO-O-) in the repeating unit, while PDO also has an additional ether group (-C=O). Unlike PDO, which is synthesized chemically by an ester condensation reaction, P4HB is currently produced by means of a recombinant Escherichia coli K12 fermentation process, followed by isolation and purification (Le Meur et al. 2013). Hydrolytic degradation of PDO and P4HB monofilament sutures have been studied both by in vitro incubation and by in vivo implantation (Ray et al. 1981; Odermatt et al. 2012). In both conditions, the primary degradation mechanism of PDO and P4HB occurs through bulk and non-enzymatic hydrolysis.

![Bond-line structure of PDO and P4HB](image)

Figure 4-1. Bond-line structure of (a) PDO and (b) P4HB (vertex: carbon and attached hydrogens, methylene group: -CH$_2$-, ester group: -CO-O-, ether group: -C=O)

The hydrolysis of semi-crystalline polymers occurs generally in a two-step sequential degradation process: first polymer chains breakdown in the amorphous regions, followed by hydrolysis in the crystalline lamella (Sabino et al. 2004). The hydrolysis is initiated by the diffusion of water molecules into the amorphous regions, followed by random chain scission of ester and other water-sensitive bonds in the polymer backbone (Gajjar & King 2014). Long polymer chains are converted to shorter fragments, which results in a reduction in polymer molecular weight. As more and more chains are cleaved to shorter fragments, the mechanical
integrity is reduced which leads to a loss in mechanical strength. When the decomposition products are even smaller and soluble in water, morphological changes, such as the formation of pores and cracks in the bulk structure, result in a rapid weight loss.

The hydrolytic degradation studies of PDO and P4HB traditional monofilament sutures have been undertaken previously and separately. However, little information is known about the effect of hydrolytic degradation on the property changes of barbed surgical sutures. The mechanical barbing process creates a plurality of barbs along the suture, which also increases the surface area with the surrounding tissue environment. This may lead to a faster rate of degradation for barbed sutures compared non-barbed ones. The structural integrity of the barb is also an uncharted topic to be addressed. The present study is the first to compare the hydrolytic degradation of PDO and P4HB barbed sutures and non-barbed sutures over a period of 10 weeks, with a focus on the changes to mechanical properties and barb morphology.

4.2 Materials and methods

The hydrolytic degradation of PDO and P4HB barbed and non-barbed sutures was conducted according to ASTM F-1635 Standard Test Method for in Vitro Degradation Testing of Hydrolytically Degradable Polymer Resins and Fabricated Forms for Surgical Implants.

4.2.1 Suture samples

Size 2-0 PDO and P4HB monofilaments were cut into 55-cm long segments. Barbed sutures were fabricated with barbs having a 165° cut angle and a 30% cut depth using the mechanical barbing machine described in Chapter 3. A total of 328 suture samples were prepared for the 10-week hydrolytic study. From Week 0 (control group) to Week 10, a total of 11 time points were included in the experiment. The sample size for each test is listed in Table 4-1. For the
nondestructive measurement of suture diameter and weight, five replicates were prepared in each suture sample group for 11 time points. A different number of knots (red arrows in Figure 4-2) were tied to differentiate suture replicates for later repeated measurements. For the tensile test, five replicates were prepared in each suture sample group per time point. For the DSC/SEM/optical microscopy tests, two replicates were prepared in each suture sample group per time point.

<table>
<thead>
<tr>
<th>Test</th>
<th>Suture sample groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDO-N</td>
</tr>
<tr>
<td>Diameter/weight measurement for 11 time points</td>
<td>5</td>
</tr>
<tr>
<td>Tensile test for each time point</td>
<td>5</td>
</tr>
<tr>
<td>DSC/SEM/Optical microscopy for each time point</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>$5 + 5 \times 11 + 2 \times 11 \times 4 = 328$</td>
</tr>
</tbody>
</table>

### 4.2.2 Degradation conditions

The suture segments were immersed in 25 ml phosphate buffered saline (PBS) solution at pH 7.4 (Gibco®, 1X) in a 2 oz. glass jar as shown in Figure 4-2. All samples were stored at a physiologic temperature of 37°C with agitation in an incubator shaker (C-24 Classic Benchtop Incubator Shaker, Edison, NJ) shaking at 50 rpm. The pH of the PBS medium was monitored every day using Accumet AB15 Basic pH meter. To prevent the accumulation of acid products and the pH dropping below 6, the hydrolytic medium was refreshed when needed. Suture samples were removed weekly for a series of measurements over a 10-week period. Suture samples that were
not exposed to these hydrolytic conditions were included as a control group and labeled as Week 0. One group of PDO sutures was able to undergo hydrolytic degradation for 150 days.

Figure 4-2. PDO barbed and non-barbed suture samples immersed in PBS. (Arrows point to knots used to distinguish replicates)

4.2.3 Measurement of weight and diameter

At each desired time point, suture samples prepared for the nondestructive measurement were removed from the PBS and dried using Kimwipes. The diameter of the suture samples was measured at the non-barbed section using an IDU 25E thickness gauge (S.D.L. International Limited, England). After drying overnight in a vacuum desiccator, the weight was measured using an Ohaus Adventurer™ Pro Precision balance with an accuracy of 0.0001 g. Since weight measurement is a non-destructive analysis, suture samples were returned to the glass jar for additional hydrolytic degradation.

4.2.4 Measurement of tensile properties

Mechanical tensile tests were performed on the suture samples in the same manner as mentioned in Chapter 3. The average tensile properties such as maximum tensile force, strain at break, stiffness and toughness (work to rupture) were calculated from the load-displacement curves of 5 replicates in each suture type group.
4.2.5 Differential scanning calorimetry (DSC)

In order to track the effect of hydrolysis on the crystalline microstructure of the suture samples, thermal analysis was also performed at 11 time points using the same method as described in Chapter 3.

4.2.6 Gel permeation chromatography (GPC)

The molecular weight and polydispersity index of P4HB from different degradation periods were analyzed using the same method as described in Chapter 3. The GPC test was not performed on the PDO samples due to the difficulties of dissolving the polymer in THF. Changes in the molecular weight of PDO were obtained from a previous study (Sevrin et al. 2015).

4.2.7 Optical microscopy (OM) and scanning electron microscopy (SEM)

Changes in surface morphology of the suture samples during the period of hydrolytic degradation were observed using a Nikon optical microscope under 20x and 100x magnification, and using a Hitachi S3200N variable pressure SEM with magnifications of 200x and 2000x. The suture samples for SEM were sputter-coated with gold/palladium and observed under an electron beam with a 5 kV accelerating voltage.

4.2.8 Statistical analysis

The effect of suture material, barb presence, and degradation time on the tensile properties of the suture samples was analyzed by a three-way ANOVA using JMP Pro 13. Specific pairwise comparisons were made using the least square means and Tukey-Kramer HSD posthoc analysis. Differences were considered significant at p < 0.05.
4.3 Results

4.3.1 Renewal of hydrolytic medium

The hydrolytic medium was refreshed five times for the PDO sutures, with the first renewal at Week 7, second and third renewal at Week 8, fourth renewal at Week 9, and the fifth renewal at Week 10. For the extended degradation up to 150 days, the medium was refreshed every other day. The only time of medium renewal for the P4HB sutures occurred at Week 4, when the pH value exceeded 7.4. No more renewal was required for P4HB during the rest of the 10-week degradation study.

4.3.2 Weight loss and change of diameter

The initial average weight of PDO barbed and non-barbed sutures was 80.6 mg and 80.3 mg, respectively. The initial average weight of P4HB barbed and non-barbed sutures was 67.6 mg and 62.9 mg, respectively. Weight loss of the suture samples during hydrolytic degradation was measured in terms of weight retention (Figure 4-3), which was calculated as the ratio of the average weight of the suture samples at a specific week compared to that of the control samples at Week 0.

\[
\text{Weight retention} (\%) = \frac{\text{Weight at week } i}{\text{Weight at week } 0} \times 100\% ,
\]

in which \( i \) has a value between 0 and 10.
During the 10-week hydrolysis study, significant differences in weight retention were observed between the PDO and P4HB sutures, whereas no significant differences between the barbed and non-barbed sutures. The PDO sutures slowly lost weight from Week 1 to Week 7, followed by a rapid weight loss during the last three weeks. By the end of the hydrolytic degradation study, the PDO sutures lost 6.5% of the initial weight, while P4HB sutures had no apparent weight loss.

The change in suture diameter within the non-barbed section is shown in Figure 4-4. No significant differences were found either between the two materials or among the different degradation times.
4.3.3 Change of tensile properties

Typical load-displacement curves of PDO and P4HB barbed and non-barbed suture samples for each week of hydrolysis are shown in Figure 4-5. The maximum tensile load and the elongation at break of the PDO sutures gradually decreased as a function of degradation time, whereas the changes to the P4HB sutures were small and less significant. The tensile testing for the PDO barbed suture samples was only possible for the first 8 weeks, because the suture samples became too weak to be mounted on the tensile tester at Weeks 9 and 10.
(a) PDO non-barbed sutures  
(b) PDO barbed sutures  
(c) P4HB non-barbed sutures  
(d) P4HB barbed sutures  

Figure 4-5. Typical tensile load-displacement curves for each week of hydrolysis

4.3.3.1 Maximum tensile force

The maximum tensile force of the PDO barbed sutures at Week 0 was 30.31 ± 1.58 N, which is about 58% of the value for the non-barbed suture (52.21 ± 2.89 N). With respect to the P4HB barbed sutures, the maximum tensile force started at 30.62 ± 3.04 N at Week 0, which is about 30% of that for the non-barbed sutures (101.96 ± 2.87 N). The loss of maximum tensile force for the barbed sutures compared to the non-barbed ones is likely primarily due to the reduction in
the effective cross-sectional area after the barbing process. Since P4HB barbed sutures had a higher reduction in cross-sectional area due to the deeper cut depth, the suture strength loss for the P4HB was greater than for the PDO barbed sutures. At Week 0, PDO and P4HB barbed sutures had equivalent values for their maximum tensile force. However, the P4HB suture in its original non-barbed state had a significantly higher tensile force than the PDO non-barbed sutures. Changes in the maximum tensile force (N) and strength retention (%) are plotted as a function of hydrolysis time in Figure 4-6, which shows a distinctly different degradation profile for the PDO sutures compared to the P4HB sutures. The maximum tensile force for the PDO sutures decreased continuously with longer hydrolytic degradation time, and by the end of the hydrolysis study little strength remained at Weeks 9 and 10. On the other hand, the P4HB sutures maintained more constant strength retention values between 70% and 90% of the initial maximum tensile force during the 10-week hydrolysis. Significant differences in strength retention (p < 0.0001) were found between the PDO barbed and non-barbed sutures, with the barbed sutures having a significantly faster drop in maximum tensile force. No significant difference (p = 0.666) was found between the P4HB barbed and non-barbed sutures.
Figure 4-6. Changes in (a) maximum tensile force and (b) strength retention as a function of hydrolysis time

4.3.3.2 Strain at break

The percent strain at break was calculated as the elongation at break divided by the initial gauge length of 200 mm. Changes in the strain at break for the two materials (Figure 4-7) followed
a similar trend to the changes in strength retention. The PDO sutures exhibited a continuous decrease in strain at break, whereas the P4HB sutures maintained a steady value, except for an increase for the P4HB barbed sutures at Week 4, and this increase was maintained until Week 10. Clearly the presence of barbs in both materials significantly reduced the values for strain at break since the barbed sutures invariably broke at a significantly lower strength than the non-barbed sutures.

![Figure 4-7. Changes in strain at break as a function of hydrolysis time](image)

4.3.3.3 Stiffness

The stiffness was measured as the initial linear slope of the load-displacement curve. Changes in stiffness as a function of degradation time are plotted in Figure 4-8. Unlike the declining trend of strength retention and strain at break, an increase in stiffness was observed for the PDO sutures. The stiffness of the PDO non-barbed sutures was lower than that of the P4HB non-barbed sutures for the first three weeks, and then it surpassed the stiffness of the P4HB sutures from Week 4 onwards. The stiffness of the P4HB sutures exhibited much variation, especially for
the P4HB barbed sutures during the first three weeks, which coincides with the variability of the load-elongation curves illustrated in Figure 4-5 (d).

![Figure 4-8. Changes in stiffness as a function of hydrolysis time](image-url)

4.3.3.4 Toughness

The toughness, also known as the work to rupture, was calculated as the area under the load-displacement curve. The changes in toughness shown in Figure 4-9 indicate that a continuous decrease in toughness was observed for the PDO sutures, while the toughness of the P4HB sutures remained constant during the 10-week degradation study. The barbed sutures had a significantly lower toughness compared with the non-barbed sutures regardless of the suture material.
4.3.4 Differential scanning calorimetry (DSC)

The DSC thermograms for PDO and P4HB as a function of hydrolysis time are shown in Figure 4-10. At Week 0, both materials had two endotherm peaks. However, the two peaks for PDO were side by side at 100 °C and 107 °C, and the two peaks for P4HB were further apart at 61 °C and 73 °C. The two separate peaks of PDO were observed for the first three weeks and then merged into one broad peak at Week 4. The higher temperature peak of P4HB moved quickly towards the lower peak and vanished at Week 3. The melting temperature of both materials increased during the 10-week period of hydrolytic degradation as shown in Figure 4-11 (a). The $T_m$ for PDO increased rapidly during the first four weeks, followed by a gradual increase in temperature during the rest of the hydrolysis period. For P4HB, except for a sharp rise during Week 1, a gradual increase in melting temperature was observed during the remainder of the 10 week study. Changes in the heat of fusion appear to be considerably different between the two materials, as seen in Figure 4-11(b). PDO showed a steady increase in its heat of fusion with longer degradation times, while the heat of fusion of P4HB fluctuated between 60 J/g to 70 J/g over the
10 week time frame. The heat of fusion of 100% crystalline PDO is 140.8 J/g, which means that the level of crystallinity of the PDO sutures increased from 57% to 74% by the end of the hydrolytic degradation study. Since the heat of fusion for P4HB is not available, its crystallinity could not be calculated.

Figure 4-10. DSC thermograms of PDO (left) and P4HB (right) as a function of hydrolysis time

Figure 4-11. Changes in melting temperature and heat of fusion as a function of hydrolysis time
4.3.5 Gel permeation chromatography (GPC)

The average molecular weight of P4HB gradually decreased during the 10 weeks of hydrolytic degradation, as shown in Figure 4-12(a). P4HB had an initial average molecular weight of 315,000 Daltons at Week 0, and experienced a 18.6% reduction in molecular weight during the 10 weeks of hydrolysis. The polydispersity index (PDI) of P4HB stayed constant around 2.0 for the duration of the hydrolysis study.

![Figure 4-12](image)

Figure 4-12. Changes in (a) average molecular weight and (b) polydispersity index of P4HB as a function of hydrolysis time (* Data replotted from Sevrin (Sevrin et al. 2015))

4.3.6 Optical microscopy and scanning electron microscopy (SEM)

Optical microscopy images and SEM images of the barbed suture samples were taken each week after drying overnight in a vacuum desiccator. The results from Week 0, 1, 4, 7 and 10 are shown in Tables 4-2 and 4-3. Long, straight grooves, left behind by the cutting tool of the barbing machine, were observed underneath the barbs. Gradual fading of the violet color from the PDO sutures was observed at longer degradation times. The integrity of the barbs was well maintained for both materials during the 10-week long period of hydrolysis. A series of blade marks was left
on the inside of the barb’s cut surface. The roughness of the cutting surface for the PDO barbs was greater than for the P4HB barbs, due to the presence of small particles in the polymer.

Table 4-2. Optical microscopy images of a single barb

<table>
<thead>
<tr>
<th>Week</th>
<th>PDO</th>
<th>P4HB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20X</td>
<td>100X</td>
</tr>
<tr>
<td>Scale bar</td>
<td>1mm</td>
<td>200 μm</td>
</tr>
<tr>
<td>0</td>
<td><img src="image1.png" alt="Image" /></td>
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</tr>
<tr>
<td>1</td>
<td><img src="image5.png" alt="Image" /></td>
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</tr>
<tr>
<td>4</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
<tr>
<td>7</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
</tr>
<tr>
<td>10</td>
<td><img src="image17.png" alt="Image" /></td>
<td><img src="image18.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Table 4-3. Scanning electron microscopy images of a single barb

<table>
<thead>
<tr>
<th>Week</th>
<th>PDO</th>
<th>PDO</th>
<th>P4HB</th>
<th>P4HB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200X</td>
<td>2000X</td>
<td>200X</td>
<td>2000X</td>
</tr>
<tr>
<td>Scale bar</td>
<td>200 µm</td>
<td>20 µm</td>
<td>200 µm</td>
<td>20 µm</td>
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</tr>
<tr>
<td>1</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td><img src="image7" alt="Image" /></td>
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<tr>
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<td><img src="image18" alt="Image" /></td>
<td><img src="image19" alt="Image" /></td>
<td><img src="image20" alt="Image" /></td>
</tr>
</tbody>
</table>

For the PDO barbed sutures, many needle-shaped fibrils were observed on the suture surface (Figures 4-13 and 4-14). The chemical composition of the fibrils was confirmed by backscattered electrons to be the same as the main body of the suture. Longitudinal grooves and
cracks appeared at Week 7. These longitudinal cracks propagated in the axial direction and created a very curly and fragile structure, especially when removed from the hydrolysis medium (Figure 4-15).

Figure 4-13. Optical microscopy images of PDO sutures at Week 6 showing the surface covered randomly by needle-shaped fibrils

Figure 4-14. Scanning electron microscopy images of PDO sutures at Week 10 showing longitudinal cracks and the surface covered randomly by needle-shaped fibrils

Figure 4-15. Optical microscopy images of PDO sutures at Week 10 showing the formation of longitudinally oriented cracks and leading to broken ends.
For the P4HB barbed sutures, small pores (Figure 4-16) were observed at Week 3 on the cut surface of the barb. The size, frequency and distribution of these pores did not change during subsequent hydrolysis exposure.

Figure 4-16. Small pores were observed on the barb cut surface of the P4HB suture at Week 5

The hydrolytic degradation study of PDO barbed sutures was extended up to 15 weeks. The suture samples tended to crumble when manipulated with tweezers. Only morphological images were taken using optical microscopy and SEM. In addition to the longitudinal cracks observed on the suture surface, multiple transverse cracks appeared on the suture’s surface as well as on the inner surface of split or barbed sutures as shown in Figure 4-17.
Figure 4-17. Multiple transverse cracks observed on (a) the suture surface and (b) on the inner surface of a split PDO barbed suture at Week 15.

4.4 Discussion

In general, changes in the appearance, physical and chemical characteristics of the PDO sutures differed from those for the P4HB sutures in a number of respects over the 10-week hydrolysis study. The PDO sutures had a more rapid loss of morphological integrity, mechanical stability and mass compared to the P4HB sutures, which confirms that PDO has a more rapid degradation rate than P4HB. These disparities caused by the different degradation rates of PDO
versus P4HB can be attributed to the distinct difference in chemical structure, molecular weight, and the presence of the ether bond. The average molecular weight of P4HB is at least twice as high as that of PDO, despite the variability in initial molecular weight of PDO monofilaments available on the market (Sevrin et al. 2015). The high molecular weight of P4HB is associated with fewer end groups and a reduced free volume, which is likely to reduce the penetration of water molecules into the semi-crystalline structure and increase the resistance of molecular chain movement and polymer chain breakdown. The absence of ether bonds in P4HB reduces the polar sites that water molecules can attack. For this reason, bulk hydrolysis of the chemical bonds in PDO occurs more rapidly leading to scission of the polymer chains at the hydrolytically unstable ester bonds, and the subsequent formation of smaller soluble segments. The reason for the difference in average molecular weight can be traced back to the different synthesis approaches, as PDO is synthesized by a chemical condensation reaction, while P4HB is synthesized by means of bacterial fermentation.

The loss of mass for the PDO sutures after 10 weeks of hydrolysis was 6.5%. But this loss of mass did not occur uniformly in a linear function with time. A more rapid drop in mass was observed after Week 7. At the same time, there was no significant change in suture diameter, indicating that bulk erosion rather than surface erosion was the most likely mechanism. This erosion kinetics resulted from a faster rate of material degradation compared with the rate of water diffusion. The acidic by-products, which accumulated by Week 7, may have been responsible for the accelerated hydrolysis triggered by an autocatalytic mechanism during the last 3 weeks. In the case of the P4HB sutures, no significant changes were observed in either the suture mass or the suture diameter.
PDO had a significant loss of maximum tensile force, strain at break and toughness between Week 3 and Week 4, which was consistent with the results reported in the literature (Lin, Chu, and Grubb 1993; Ooi and Cameron 2002b). By the end of the 10-week hydrolysis study, the PDO sutures had almost no strength left, while the P4HB suture retained at least 70% of its initial strength. The constant suture mass and limited loss in tensile properties indicated that the degradation for P4HB was still in an early stage. The slower rate of hydrolysis of P4HB could also be inferred by the fact that it only required one change of hydrolysis media, compared with five times for the PDO sutures.

The melting temperature for both materials increased during the 10-week hydrolysis study. In addition, an increase in the heat of fusion and the level of crystallinity were also observed, but only for the PDO sutures. These increases in melting temperature and heat of fusion for PDO sutures have been reported previously (Lin, Chu, and Grubb 1993; Ooi and Cameron 2002b; Sabino et al. 2000). It is believed that random chain scission occurred first in the amorphous regions, which reduced the entanglement of the polymer chains, allowing them to reorder themselves so as to produce a larger range of crystal sizes and stabilities (Lin et al. 1993; Ooi & Cameron 2002a). Hydrolytic degradation might also remove the less perfect crystals that melt at a lower temperature and hence remove the endotherm peak at the lower temperature (Sabino et al. 2000). The increase in crystallinity was also reflected by the increase of suture stiffness during tensile testing. The increased melting temperature of P4HB indicates the formation of bigger crystals that might be attributed to a recrystallization process initiated by the residual stress left in the partially drawn monofilaments (Sauer et al. 2000). The partially drawn status can be verified by a two-phase load-displacement curve (Figure 4-5 (c)), in which a load is applied to partially draw the fibers, and realign the polymer chains, which transforms a fiber with low stiffness into a
fully drawn polymer chain with higher stiffness. However, the chain movement in the recrystallization process and the simultaneous degradation process may well be offset so that the level of crystallinity for the P4HB sutures remains constant over the 10-week period.

The rapid degradation rate of the PDO suture caused a loss of morphological integrity together with fading of the purple color. Previously it has been reported that the migration of the dye molecules or pigment particles increases with hydrolysis time and medium temperature (Lin et al. 1993). A 0.2% weight of dye molecules or pigment particles had no appreciable effect on the nucleation and crystallization properties of freshly prepared PDO and PDO copolymer films (Andjeli et al. 2001; Jamiolkowski et al. 2000). In fact, the effect of this weight percent of dye molecules or pigment particles on the performance properties of surgical suture is not known. If the weight percent of the dye molecules or pigment particles was larger than 0.2%, it might have contributed to the weight loss of the PDO during the hydrolysis study. The free space left by the pigment particles may have facilitated the free movement and reorientation of the polymers chains, which might have contributed to the increased level of crystallinity. Needle-shaped fibrils were shed from the main body of the PDO sutures, followed by the formation of longitudinal cracks and the splitting of the monofilaments towards the end of the hydrolytic degradation study. The splitting of PDO monofilaments has also been reported in other studies (Wang & Zhang 2016; Sevrin et al. 2015). However, another morphological change was observed as transverse cracks appeared on the suture surface and propagated inside the sutures (Sabino et al. 2000). This new and different observation may have resulted from a different manufacturer of PDO monofilaments. The PDS II sutures used in Sabino’s study may have experienced an additional heat-setting treatment by passing the monofilaments through a tube furnace at a temperature higher than the melting temperature (Ephraim 1994). This approach was applied to reduce the modulus of the
monofilament and improve the flexibility and handling properties of the PDO sutures. Therefore, the PDS II sutures are likely to have a sheath-core structure with a less crystallized outer annular region and highly crystalline core, which was shown in the SEM images of Sabino’s study. The geometric integrity of a single barb fabricated on the PDO monofilaments was well maintained during not only the 10 weeks, but even after 15 weeks of this hydrolysis study as shown in Figure 4-17 (b).

At the same time the morphological integrity of the P4HB sutures was well maintained, other than the formation of small pores on the cut surface, but not on the monofilament’s outer surface. Surface erosion of a P4HB suture has been observed, but mainly in the degradation process mediated by enzymes, such as lipase and PHB depolymerase (Su et al. 2003). Surface erosion seen as pores on the cut surface here could be explained by the inner less crystalline structure of the monofilament being exposed to the hydrolysis media. This is especially true for P4HB, which is hard to crystallize with such a high molecular weight (Boesel et al. 2014). It was thought that the monofilaments manufactured by melt spinning might have a different microstructure across the radius, since the outer surface of the monofilaments would have been in direct contact with the cooling air below the spinneret causing rapid cooling and alignment of the polymer chains at and near the outer surface. This outer solidification would limit the mobility of the polymer chains at the center or core of the monofilaments, and so they would form a semi-crystalline structure with a less ordered structure. However, this is only a speculative assumption! No one has yet proven this. The formation of these internal pores might have influenced the tensile properties of the P4HB barbed sutures, which resulted in an increase in strain at break and a decrease in suture stiffness.
Changes in the mechanical and tensile properties of sutures during degradation are related to the chemistry and processing of the suture material as well as the barbing process. The barbing process caused a marked decrease in the effective cross-sectional area of the monofilaments. This generated considerable impact on the material’s mechanical properties, as well as a lesser impact on the rate of degradation. When the barbed sutures were compared with their non-barbed equivalent, the maximum tensile force, the strain at break and the toughness were all significantly reduced. The barbing process reduces the effective area for load bearing, while at the same time, creating new surfaces and enlarging the surface area. There were concerns about whether the new generated surface area would accelerate the degradation rate of absorbable polymers. In this study, it was found that the rate of change for both weight and mechanical properties were identical for the barbed and the non-barbed sutures. However, this study also focused on changes in the surface morphology of single barbs. Small pores were observed on the cut surface of the P4HB barbed sutures, indicating that the newly exposed cut surface may have changed the degradation mechanism from bulk to surface erosion.

A suture material’s intrinsic properties have a great influence on the barb geometry and clinical performance. As mentioned in Chapter 3, the percentage of the original cross-sectional area of barbed sutures is reduced more when the cut depth goes deeper than 25% of the suture diameter. So having set-up the machine to cut 30% cut depth, the PDO barbed sutures were found to have a 31% average cut depth and 24% of the original cross-sectional area left, whereas the P4HB barbed sutures were found to generate a 36.5% average cut depth with only 12% of the original cross-sectional area remaining. This is the major explanation for the significantly higher strength loss of P4HB barbed sutures than PDO barbed sutures due to the mechanical barbing process.
It should be noted that this study has been primarily concerned with the effect of hydrolytic degradation on the mechanical and morphological properties of PDO and P4HB barbed and non-barbed sutures. Other mechanisms will operate when the hydrolysis is enzymatically initiated. So the results reported here should not be interpreted to apply to conditions where there is enzymatic hydrolysis.

4.5 Chapter Summary

For the first time, a hydrolytic degradation study has been applied to PDO and P4HB barbed and non-barbed sutures. Due to the high molecular weight synthesized during bacterial fermentation, P4HB was found to have more resistance to hydrolysis compared to PDO. The rapid degradation rate of PDO resulted in a quick loss of mechanical stability and morphological integrity during the 10-week hydrolysis study. The barbing process had a significant impact on the suture’s tensile strength due to the reduction in effective cross-sectional area. It is worth noting that the increased surface area created by the barbing process had no significant influence on the degradation rate. As a result of this study, further studies could be undertaken to measure the effect of the barbed suture’s surface area on the morphological changes experienced by the barbed suture geometry.
CHAPTER 5: FACTORS INFLUENCING THE ANCHORING PERFORMANCE OF BARBED SURGICAL SUTURES

5.1 Introduction

Barbed surgical sutures have been applied to various fields, such as cosmetic, plastic, orthopedic, urological, gynecological and other types of surgeries (Lin et al. 2016). The advantages of using barbed surgical sutures include introducing a more uniform distribution of residual tension, the elimination of bulky knots, improved suturing efficiency and enhanced interactions with the surrounding tissues. In summary, a higher anchoring performance is the essence of the superior clinical outcome for barbed sutures. Many factors have been reported to influence the anchoring performance of barbed sutures, such as barb geometry and tissue type. In order to achieve the best mechanical anchoring with skin tissue, the barbs should be flexible with a long barb length; while for tendon tissue, the barbs should be stiff with a deeper cut depth (Ingle & King, 2010). The needle swaged onto the barbed suture also has potential effects on the interaction between the barbs and the surrounding tissue. A needle with a large diameter and sharp needle point may disturb the structural integrity of the tissue and limit the barb’s ability to penetrate and anchor the surrounding tissue (Ruff 2006a). In addition to the barb geometry, tissue type and needle type, other factors, such as suture material, suture size and testing method may also affect the anchoring performance of barbed sutures. Several testing methods have been published to measure the anchoring performance of barbed sutures or to compare their performance with conventional knotted sutures. The first method is the suture/tissue pullout test, in which the barbed suture is first inserted in a semi-circular pattern within the thickness of a tissue or tissue simulant specimen, and is then pulled back out. The anchoring performance is measured as the maximum pullout force (Ingle & King, 2007; Ruff, 2006). The second method is the wound closure test, in
which a wound is closed with barbed sutures, and then the approximated tissue is loaded in the transverse direction until the wound opens. The anchoring performance is measured as the force required to generate a 2-mm gap (Zaruby et al. 2011; Templeton et al. 2015; Shimizu et al. 2017; Law et al. 2017). A third rumpled skin method has been used to quantify the anchoring performance of barbed sutures by measuring the change in length of rumpled skin over time (Jang et al. 2005). A fourth photoelastic method has been used to characterize the anchoring performance by measuring the number, magnitude and shape of the fringe distribution along the length of the suture (Hoy & Gingras 2015). The suture/tissue pullout test and wound closure test have been used more often than the rumpled skin or photoelastic methods. However, as yet there is no established standard test method to measure the anchoring performance of barbed sutures. The focus of the current study is to discover the effects of five different factors on the in vitro anchoring performance of barbed sutures in porcine skin, and to establish the degree of correlation between the anchoring performance measured by the pullout test and the wound closure test.

5.2 Materials and methods

5.2.1 Description of experimental factors

Five experimental factors were included in the study, namely, suture material, barb geometry, suture size, swaged needle type and tissue type. Each factor had two levels except for suture size, which had three levels.

5.2.1.1 Suture material

Barbed sutures fabricated with PDO and P4HB monofilaments were fabricated using a mechanical barbing machine as described in Chapter 3.2. Two different suture lengths were fabricated for the suture/tissue pullout test and wound closure test as shown in Figure 3-20 (b – c).
Sutures used for the suture/tissue pullout test were unidirectional barbed sutures with a 3.5 cm barbed section, while sutures used for the wound closure test were bidirectional barbed sutures with two 7 cm barbed sections and a non-barbed transitional section measuring 0.5 cm in between. In addition to the experimental PDO and P4HB barbed sutures, two commercial barbed suture products, size 2-0 Quill™ PDO and size 2-0 Monoderm™ were included in the suture/tissue pullout test for comparison purposes.

5.2.1.2 Barb geometry

Barbs were cut on the monofilaments using the mechanical cutting machine described in Chapter 3.2. The length of the barb was changed from short to long by simply flipping the cutting blades. A side view of the blade and the corresponding barb geometry are shown in Figure 5-1. The average cut depth, cut angle and cut length of the short barbs were 31%, 165° and 457 μm, while the geometry of long barbs was 24%, 173° and 748 μm.

![Figure 5-1. Short (left) and long (right) barb geometries generated by flipping the cutting blade](image)

5.2.1.3 Suture size

Three suture sizes, namely, size 0, 2-0 and 3-0 were selected. The average diameter for each size was 467 μm, 366 μm and 298 μm.
5.2.1.4 Needle type

Curved needles (3/8 circle) obtained from B. G. Sulzle Inc. (Syracuse, NY) were swaged onto the fabricated barbed sutures using a needle swaging machine. The needles had either a taper point or a diamond point shown in Figure 5-2. The needle size was selected to match the suture size, and the diameter of the needle was about 1.6 times as big as the diameter of the suture.

![Figure 5-2. Swaged needle points: (a) taper point and (b) diamond point](image)

5.2.1.5 Tissue type

Dorsal skin tissue was harvested from female pigs weighing 25-30 kg from the College of Veterinary Medicine, North Carolina State University. The harvested skin tissue was wrapped with cotton gauze soaked in PBS and stored at -10°C in the freezer for future testing. Before performing the suturing and anchoring test methods, the frozen skin tissue was thawed overnight at room temperature. The tissue type, epidermis or dermis, was determined by which side the barbed suture was inserted. A preliminary test showed that there was no significant difference in the maximum pullout force measured on fresh skin tissue, thawed frozen tissue and frozen tissue hydrated with PBS for 2 hours (p = 0.4115). The average maximum pullout force of hydrated
frozen tissue was similar to that for fresh tissue. So 2 hours of immersion in PBS was applied after thawing overnight.

5.2.2 Testing methods for anchoring performance measurement

5.2.2.1 Suture/tissue pullout test

In the suture/tissue pullout test, unidirectional barbed sutures were inserted into the skin tissue following a pre-determined suturing pattern. An embroidery hoop with a diameter of 10 cm was used to help stretch and flatten the skin tissue. A schematic diagram of the pullout test, including the suturing pattern, tissue mounting and pullout direction, is shown in Figure 5-3. In order to keep the length of the engaged barbed suture constant among different samples, a three-point marker was made on the skin tissue to indicate the enter and exit points for the barbed sutures. Two stitches were inserted to create a semi-circular curved pattern, which resulted in a barb-engaged length of about 2 cm. One PDO barbed suture and one P4HB barbed suture were inserted side by side. A pair of customized U-shaped clamps were made to clamp the three edges of the flexible skin tissue. The wavy teeth shown in the side view were designed to increase the friction and clamping pressure, and prevent slippage of the skin tissue. The entire specimen was mounted on an Instron Model 5544 mechanical tester (Canton, MA) equipped with 100 N load cell. The barbed suture was pulled out at a rate of 20 mm/min in the opposite direction to the insertion direction. The maximum pullout force (N) was recorded by the Bluehill® Software as the anchoring performance of the barbed suture. After pulling out the sutures from the skin tissue, the barbed sutures were examined under a magnifying glass to record the type of failure mode as either barb bending, barb peeling or suture breakage.
5.2.2.2 Wound closure test

In the wound closure test, bidirectional barbed sutures were used to close an incision. A schematic diagram of the of wound closure test method, including the tissue dimensions and clamping, the suturing pattern and the direction of tensile loading, is shown in Figure 5-4. A straight line measuring 4-cm long was drawn in an anterior / posterior direction on the lateral region of the pig. Nine markers, 5 mm distance apart, were added to the straight line as guidance for the needle entering and exit points. A transection was made on the skin tissue through the entire thickness following the straight line. The incision was closed using a bi-directional barbed suture with the initial needle insertion point starting at the middle of the incision. A curved suturing pattern similar to that in the suture/tissue pullout test was used; however, the semi-circular curve was completed within one stitch. Suturing started with the curved needle entered from the subcutaneous layer. It then went up through the dermal layer to the epidermal layer and came back to the subcutaneous layer as shown in Figure 5-5. After applying a tension to the suture, a little dent appeared on top of the skin tissue, which confirmed that anchoring had been generated between the barbs and the surrounding tissue. The suturing was continued with a simple running stitch and two backup stitches at the end. Suturing was completed by repeating the same procedure.
with the other arm of the barbed suture running in the other direction. PBS was sprayed on the skin tissue to prevent dehydration.

Figure 5-4. Schematic diagram of wound closure test

Figure 5-5. Dr. Gregory L. Ruff’s drawing of the suturing method
The sutured skin tissue was mounted on the Instron mechanical tester using a pair of flat clamps. The specimen was pulled to failure in a rate of 20 mm/min. The ultimate load to failure was recorded as the anchoring performance of the barbed suture, and the type of failure was recoded as either a barb failure (suture slippage in the tissue), suture breakage or a tissue tear (suture tearing through the skin tissue without breaking).

In addition to the ultimate load to failure, the force required to generate a 2-mm gap was also measured using a video camera and modeling software called “Tracker”. The entire biomechanical testing procedure for each sample was recorded simultaneously with the Instron mechanical tester and a Canon HD camcorder. The recorded video with 30 frames per second was uploaded to the Tracker software. A calibration stick measuring 2 mm long was created, based on the reference ruler in the video. The gap of the wound dehiscence was examined frame by frame until it reached 2 mm. The time when the 2-mm gap was generated was used to track the corresponding load in the tensile test raw data file.

Figure 5-6. 2-mm gap analysis using Tracker software
5.2.3 Experimental design

All five factors, namely, suture material, barb geometry, suture size, swaged needle type and tissue type were included in the measurement of anchoring performance using the suture/tissue pullout test. Two factors, suture material and suture size, were included in the design of the wound closure test. Full factorial designs were created for each testing method described as follow.

Two factorial designs, Design I and Design II, were created to discover the effects of five factors on the anchoring performance of barbed sutures using the suture/tissue pullout test. Each design had two constants and three variables as shown in Table 5-1. A total of 20 groups were involved in the study, with 8 groups in Design I and 12 groups in Design II. The sample size for each group was 6.

Table 5-1. Layout of two full factorial designs for suture/tissue pullout test

<table>
<thead>
<tr>
<th>Design</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constants</td>
<td>Long barb, size 2-0</td>
<td>Taper point needle, dermis tissue</td>
</tr>
<tr>
<td>Variables</td>
<td>Suture material</td>
<td>Needle type</td>
</tr>
<tr>
<td></td>
<td>PDO</td>
<td>Diamond Taper</td>
</tr>
<tr>
<td></td>
<td>P4HB</td>
<td>PDO</td>
</tr>
<tr>
<td>Variable levels</td>
<td>PDO</td>
<td>P4HB</td>
</tr>
<tr>
<td></td>
<td>P4HB</td>
<td>P4HB</td>
</tr>
</tbody>
</table>

Since both epidermis and dermis were included in the wound closure test, the factor of tissue type was excluded. The swaged needle type was kept as a constant at taper point. It was found from the suture/tissue pullout test that barbed sutures with short barbs had a significant higher anchoring performance than those with long barbs. Therefore, the barb geometry was also
kept as a constant at short barbs. Therefore, two variables, suture material and suture size were involved in the full factorial design for the wound closure test as shown in Table 5-2.

Table 5-2. Layout of full factorial design for wound closure test

<table>
<thead>
<tr>
<th>Design</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constants</td>
<td>Short barbs, taper point needle and full thickness of skin tissue</td>
</tr>
<tr>
<td>Variables</td>
<td>Suture material</td>
</tr>
<tr>
<td>Variable levels</td>
<td>PDO</td>
</tr>
<tr>
<td></td>
<td>P4HB</td>
</tr>
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<td></td>
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</tbody>
</table>

5.2.4 Statistical analysis

The effect of suture material, needle type, and tissue type on the anchoring performance measured in the pullout test was analyzed by a three-way ANOVA using JMP Pro 13. Specific pairwise comparisons were made using least square means and Tukey-Kramer HSD posthoc analysis. Differences were considered significant at p < 0.05. The same statistical analysis was used for the effect of suture material, barb geometry and suture size on the anchoring performance measured in the wound closure test. The correlation of anchoring performance measured between these two tests was analyzed by a Pearson correlation coefficient using JMP Pro 13. The correlation was considered significant at p < 0.05.

5.3 Results

5.3.1 Results of the suture/tissue pullout test

The results of anchoring performance measured as the maximum pullout force in Design I and Design II were plotted in Figures 5-7 and 5-8. The highest pullout force in Design I was 14.58
± 1.49 N which was observed with the PDO barbed sutures swaged with taper point needles and inserted in the porcine epidermal tissue. The highest pullout force in Design II was 13.59 ± 1.21 N which was observed for size 0 PDO barbed sutures fabricated with short barbs. The three-way ANOVA results showed that the anchoring performance of barbed sutures in the suture/tissue pullout test was significantly influenced by all five factors, namely, suture material (p < 0.0001), needle type (p = 0.0005), tissue type (p = 0.0079), barb geometry (p <0.0001) and suture size (p< 0.0001). Barbed sutures had a higher anchoring performance when they were fabricated from the larger size PDO sutures with short barbs, swaged with taper point needles and deployed in the epidermis. The anchoring performance was also significantly influenced by the three-factor interaction parameter among three variables in Design I, namely, suture material * needle point * tissue type.

![Graph showing anchoring performance of barbed sutures](image)

Figure 5-7. Anchoring performance of barbed sutures measured in Design I (size 2-0 barbed sutures with long barbs)
During the pullout test, no barbed suture breakage was observed. Images of the barb failure modes for PDO and P4HB barbed sutures are shown in Figure 5-9. The barbed sutures fabricated from PDO were pulled out from porcine skin due to the barbs bending backwards and peeling from the main body of the suture, while the P4HB barbed sutures lost their anchoring ability with the surrounding tissue mainly due to barb bending.
The anchoring performance of size 2-0 PDO and P4HB barbed sutures fabricated with long and short barbs was compared with the two commercial products. Two distinct maximum pullout forces were observed in the data plotted in Figure 5-10. The statistical results showed that PDO and P4HB barbed sutures with short barbs had an equivalent anchoring performance to the Quill™ barbed sutures, while the PDO and P4HB barbed sutures with long barbs had an equivalent anchoring performance with the Monoderm™ barbed sutures.

![Figure 5-10. Anchoring performance of PDO and P4HB barbed sutures compared with two commercial products (L: long barbs, S: short barbs)](image)

5.3.2 Results of wound closure test

The anchoring performance measured as the ultimate load to failure and the load to form a 2-mm gap in the wound closure test is plotted in Figure 5-11. The highest ultimate load to failure was measured at $89.19 \pm 14.86$ N for size 2-0 PDO barbed sutures. There were no significant differences between this load to failure and that measured in the groups of size 0 PDO barbed sutures and size 3-0 P4HB barbed sutures. The highest load to form a 2-mm gap was measured at $81.97 \pm 16.77$ N for size 0 PDO barbed sutures. There was no significant difference between this
value and that measured for size 2-0 PDO barbed sutures. The statistical analysis showed that the anchoring performance of barbed sutures measured by the wound closure test was related to the suture material and the two-factor interaction of suture material * suture size.

The three failure modes observed during the wound closure test and resulting in suture slippage in the tissue were observed as tissue tearing, suture breakage or barb failure. Most sutured wounds failed with a combination of two of these failure modes. The frequency of the different failure modes for PDO and P4HB barbed sutures is plotted in Figure 5-12. For size 0 barbed sutures, the sutured wound failed due mainly to a combination of torn tissue and barb failure (Figure 5-13(a)). The gap of wound dehiscence was enlarged by torn tissue while the edges of the wound were still held together with the barbed sutures, which corresponded to a drop in the tensile load but not the end of the test (Figure 5-14). As the suture size went down, suture breakage was observed, as illustrated in Figure 5-13(b), especially for the PDO barbed sutures. With suture breakage, the wound repair failed immediately and completely, and resulted in a sharp drop of the
tensile load as shown in Figure 5-14. For sizes 2-0 and 3-0 P4HB barbed sutures, suture slippage caused by barb failure, as shown in Figure 5-13(c) was the main reason for wound dehiscence. The tensile load fluctuated (Figure 5-14) while the suture slipped within the tissue as each barb failed in sequence until most of the suture was pulled out.

![Graph showing frequency of different failure modes for different suture sizes of PDO and P4HB barbed sutures](image)

Figure 5-12. Frequency of different failure modes for the different suture sizes of PDO and P4HB barbed sutures
Figure 5-13. Images of different failure modes, (a) tissue tear for size 0 PDO, (b) suture breakage for size 2-0 PDO, and (c) barb failure for size 3-0 PDO
5.3.3 Correlation between two test methods

The anchoring performance measured as the maximum pullout force in the suture/tissue pullout test did not correlate with the ultimate load to failure, or the load to form a 2-mm gap in the wound closure test. However, the ultimate load to failure correlated positively with the load to form a 2-mm gap. The correlation was stronger for PDO barbed sutures \( r = 0.9026, p < 0.0001 \) than for P4HB barbed sutures \( r = 0.6966, p < 0.0001 \). A linear fit was added to the scatter plot (Figure 5-15) of anchoring performance measured in the wound closure test for both PDO and P4HB barbed sutures. The load at 2-mm gap increased with the ultimate load to failure for both materials.
5.4 Discussion

The suture material had a significant influence on the anchoring performance of barbed sutures regardless of the test method used. Ingle measured the anchoring performance of barbed sutures fabricated from seven conventional suture materials using the suture/tissue pullout test in a chamois leather skin simulant and found that the maximum pullout force varied from 13 N to 21 N (Ingle & King, 2007). The differences in polymer properties between PDO and P4HB monofilaments have been studied in Chapter 3. In general, P4HB monofilaments have a significantly higher tensile strength and stiffness than PDO. However, P4HB barbed sutures have an equivalent strength to PDO barbed sutures as a result of the barbing process due to a deeper cut depth. Similarly, because of its inferior hardness, the barbs fabricated on the P4HB monofilaments were more flexible, flimsy and softer compared with the PDO barbs. The differences in material and barb properties resulted in different suture failure modes for both test methods. Finite element analysis has shown that the cut base of the barb is under tension, while the upper region behind
the barb is under compression (Ingle & King, 2007). When P4HB barbs were bearing the load from the surrounding tissues they tended to curl and bend over backwards, while the PDO barbs were stiffer and supported a higher anchoring load. When the anchoring load exceeded the extent of the anchoring ability, PDO barbs peeled away from the main body of the suture rather than just bending backwards. The energy consumed during the peeling enhanced the anchoring ability even after barb failure. The anchoring performance of barbs was reduced after barb bending and peeling, while the anchoring ability fell to zero when the barb was completely detached from the monofilament.

The correlation between barb stiffness and the failure mechanism can also be applied to the effect of barb geometry on the anchoring performance. The short barbs were stiffer and had a thicker base attached with the monofilament due to a deeper cut depth, compared with the long barbs. If the barb was treated as a lever, the length of the long barb increased the moment of the force, which made the barb easier to bend backwards. So barbed sutures fabricated with long flexible barbs had an inferior anchoring performance, similar to the Monoderm™ barbed sutures, even when inserted into flexible skin tissue.

The anchoring performance of barbed sutures measured by the suture/tissue pullout test decreased as the suture size got smaller, while the anchoring performance measured in the wound closure test were not influenced by suture size. No correlation was found between these two test methods. The pullout test is a simplified test which focuses more on the individual barb and the series of sequential steps that represent the failure mechanism (Ingle & King, 2007). The maximum pullout force improves with stiffer and thicker barbs and when the suture diameter increases. The wound closure test is more realistic of clinical use and is involved with both the tissue and the suture properties. In addition to the anchoring performance provided by the barbs, nine strands of
the suture material ran across the wound and contributed to the wound’s ultimate load to failure and the load to form a 2-mm gap. This explains why the anchoring performance measured by the wound closure test was about six times higher than that measured in the pullout test, even though the suture length and number of barbs involved in the wound closure test were only twice as many as those in the pullout test. Since there were more components involved in the wound closure test, the failure mode was more than just barb failure. Size 0 barbed sutures had a significantly higher tensile strength and could cut through skin tissue especially when there were barbs attached. When the suture diameter was reduced, the barbed suture broke before tearing through the skin tissue. Since there were only two locking stitches at the end of the wound, the barbed suture tended to slip in the skin tissue due to barb failure. The instance when the barbed suture started to slip was related to its failure mode and its anchoring performance. When the barbed suture slipped earlier during the test, the gap was formed at one end of the wound and then propagated to the other end. The anchoring performance for wounds with early slippage usually maintained a low load for a longer period of time. When the barbed suture slipped at a later time, the gap formed evenly across the length of the wound, and failure occurred when the tissue tore or the suture ruptured. The anchoring performance in this situation increased sharply before suture slippage and suddenly dropped when catastrophic failure occurred. PDO barbed sutures had a more secure anchoring ability at both ends of the wound compared with P4HB barbed sutures. Therefore, a majority of the PDO barbed sutures failed due to suture breakage or a tissue tear, while most P4HB barbed sutures failed due to early barb failure and suture slippage.

Even though the impact of needle type and tissue type was not as great as for the three other factors mentioned above, they were found to have a significant influence on the anchoring performance measured in the suture/tissue pullout test. Cutting needles attached to the
Monoderm™ barbed sutures ensured a smooth passage through the dense epidermis. At the same time, the needles may have damaged the surrounding tissue which affected the interaction and engagement by the flexible and flimsy P4HB barbs. However, this is just speculation, which needs to be studied further.

The type of tissue matrix that barbed sutures are inserted into has a great influence on the anchoring performance. A chamois leather skin simulant which was a highly fibrous structure was used previously for the pullout test (Ingle & King, 2007). Native tissues are also composed of a collagen fibrous structure, which is surrounded by the mass of extra cellular matrix. The fibrous structure in the chamois leather tended to be caught by the barbs more easily than in native tissues, which tended to generate higher anchoring forces compared to native tissues. Differences in fibrous structure and water content might be the reason why tissue type has a significant influence on the anchoring performance of barbed sutures. Barbed sutures have been applied to different types of tissues, the majority of which have been soft tissues with a wide range of tissue structures, components, thicknesses orientations and degrees of alignment. So, in order to optimize the geometry of barbed sutures, it is first necessary to identify the specific type and orientation of the tissue structure that you are planning to suture.

5.5 Chapter summary

The anchoring performance of PDO and P4HB barbed sutures have been measured using a suture/tissue pullout test and a wound closure test. The results of the suture/tissue pullout test showed that the anchoring performance of barbed sutures was significantly related to all five of the factors; suture material, suture size, barb geometry, needle point and tissue type, that were included in the current study. However, the anchoring performance measured in the wound closure
test was more related to the suture material rather than the suture size. While a linear correlation was found for the anchoring performance measured by these two test methods for PDO barbed sutures, no linear correlation was found between these two methods for P4HB barbed sutures.
CHAPTER 6: IN VIVO ANCHORING PERFORMANCE AND HISTOLOGIC EXAMINATION OF BARBED SURGICAL SUTURES IN A RAT MODEL

6.1 Introduction

In Chapter 4, P4HB barbed sutures have been studied to have improved strength retention compared to PDO barbed sutures during the 10-week hydrolytic degradation study. The barb configuration and geometry, and the surface morphology of P4HB also maintained their integrity better than the PDO barbed sutures. In Chapter 5, the anchoring performance of PDO and P4HB barbed sutures was measured using both a suture/tissue pullout test and a wound closure test. The lab fabricated P4HB barbed sutures exhibited an equivalent or even higher anchoring performance compared with the Quill™ and Monoderm™ commercial barbed sutures, even though they had a significantly lower anchoring performance compared to the laboratory fabricated PDO barbed sutures. To further explore the feasibility of P4HB being used as a barbed suture material in a live tissue environment, and to evaluate the effect of a prolonged degradation profile on the in vivo anchoring performance, an animal study using a rat model is described in the current chapter. P4HB and PDO barbed sutures were implanted in rat dorsal skin for 28 days. The inflammatory response of the surrounding tissue on the deployed barbed sutures was examined semi-quantitatively followed with a published scoring scheme (Zaruby et al. 2011).

A number of studies have been published on the assessment of biomechanical properties of barbed sutures and the inflammatory response in the surrounding tissue using animal models as shown in Table 6-1 (Jang, et al., 2005; Kurita et al., 2011; Law et al., 2017; Petrut et al., 2013; Zaruby et al., 2011; Api et al., 2015). Barbed suture materials that have been studied histologically include polypropylene (PP), PGA-PTMC-PDO copolymer (V-Loc 90™ and V-Loc 180™) and PGA-PCL copolymer (Monoderm™) in either a rat, porcine or canine model. The timeline of the
*in vivo* animal studies varied from days to months. After implantation, cellular infiltration was uniformly distributed around the entire circumference of the barbed suture, rather than being localized in one quadrant of the monofilament (Zaruby *et al.* 2011). An explanation for this was possibly due to the even tension distribution along the length of the barbed suture. As the implantation time increased, a thicker capsule and higher density of myofibroblasts were detected around the circumference of barbed sutures compared with conventional sutures during the first month post-implantation (Jang *et al.* 2005; Kurita *et al.* 2011). The initial strong inflammatory response that generated a thick capsule was replaced with collagen fibers after 3 months. This remodeling occurred faster than for the control gold suture, which gave a delayed short term reaction (Kurita *et al.* 2011). Fragmentation of absorbable barbed sutures occurred earlier than the non-barbed sutures, indicating that the increased surface area generated by the barbs accelerated the rate of hydrolysis (Zaruby *et al.* 2011). Micro-slippage of the suture caused by reduced barb anchoring ability may have stimulated a greater inflammatory response. Discrepancies were found between the various studies, which may be attributed to variability in species (Law *et al.* 2017). In addition to the inflammatory response, the function of the barbed sutures appeared to differ according to the application in different organs. Wound dehiscence was the primary complication in closing skin tissues, while the formation of adhesions was more problematic in myomectomy model closures, as well as cystotomy and gastrointestinal closures (Api *et al.*, 2015; Demyttenaere *et al.*, 2009; Petrut *et al.*, 2013).
Table 6-1. List of histological studies of barbed sutures

<table>
<thead>
<tr>
<th>Authors</th>
<th>Barbed suture material</th>
<th>Animal model</th>
<th>Experimental time points</th>
<th>Staining method</th>
</tr>
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<tbody>
<tr>
<td>Jang et al.</td>
<td>polypropylene</td>
<td>Sprague-Dawley (SD) rat skin</td>
<td>Day 14 and 21</td>
<td>H&amp;E staining Immunostaining for αSMA*</td>
</tr>
<tr>
<td>Kurita et al.</td>
<td>polypropylene</td>
<td>Wistar rat skin</td>
<td>Month 1, 3 and 7</td>
<td>H&amp;E staining Elastica van Gieson staining Immunostaining for αSMA*</td>
</tr>
<tr>
<td>Zaruby et al.</td>
<td>V-Loc 90™ Monoderm™</td>
<td>Yorkshire pig skin</td>
<td>Day 3, 10 and 21</td>
<td>Geimsa staining</td>
</tr>
<tr>
<td>Law et al.</td>
<td>V-Loc 90™</td>
<td>Hound dog skin</td>
<td>Day 3, 10 and 14</td>
<td>H&amp;E staining</td>
</tr>
<tr>
<td>Petrut et al.</td>
<td>V-Loc 180™</td>
<td>Wistar rat bladder</td>
<td>Week 3 and 6</td>
<td>H&amp;E staining</td>
</tr>
<tr>
<td>Api et al.</td>
<td>V-Loc 180™</td>
<td>SD rat Uterine horn</td>
<td>Week 6</td>
<td>H&amp;E staining</td>
</tr>
</tbody>
</table>

* α smooth muscle actin

The histological studies listed above measured the inflammatory response induced by barbed sutures in general. In most of the papers, the histological scores were determined by examining the cross-sectional view of the sutures, where tissue surrounded the circumference of the barbed suture as shown in Figure 6-1 (a). Since the barbed section and non-barbed section alternated along the suture length, the cross-sectional views may not have been cut exactly at the
barbed section. In addition, the tissue anchored underneath the barbs could not be assessed by viewing only cross-sectional sections. A longitudinal view as shown in Figure 6-1 (b) is a more extensive way to observe the shape of the barb, the tissue anchored underneath the barb, as well as the barb-tissue interaction. However until now, the longitudinal section has been published in only one paper studying fascial rejuvenation.

![Image](99x438 to 294x582)

![Image](297x438 to 514x579)

Figure 6-1. Histopathological views of barbed sutures from the literature, (a) cross-sectional view and (b) longitudinal view (Sulamanidze & Sulamanidze 2009; Zaruby et al. 2011)

The goal of the present study was to compare the in vivo anchoring performance and histological assessment of PDO and P4HB barbed sutures during a 28-day implantation study in a rat model. The anchoring performance of PDO and P4HB barbed sutures was measured in terms of the maximum pullout force during the suture/tissue pullout test. Histological analysis was taken at a series of time points to assess the inflammatory cell infiltration and the foreign body reaction. This was the first time that histological interactions between the barbs and the surrounding tissues were examined using both cross-sectional and longitudinal views.
6.2 Materials and methods

Size 2-0 PDO was obtained as Unify® cassette sutures from AD Surgical Inc. (Sunnyvale, CA, USA). Size 2-0 P4HB was obtained as MonoMax® sutures from B. Braun Medical Inc. in Spain. These PDO and P4HB monofilaments were then fabricated into barbed sutures (barbs with a 165° cut angle, 30% cut depth and 450 μm cut length) using the mechanical barbing machine described in Chapter 3. Barbed sutures were swaged with diamond point needles to ensure a smooth passage and minimal trauma to the rat skin. Twenty female Sprague-Dawley rats (average weight of 200 g) were used in this study. The rats were randomly assigned to one of four groups corresponding to the duration of implantation. Sacrifice took place on the following designated postoperative days: Day 3, Day 7, Day 14 and Day 28, which was reported to correspond to different healing stages: inflammation, fibroplasia, maturation and remodeling (Zaruby et al. 2011).

6.2.1 Deployment of suture samples

The barbed suture samples were sterilized in 75% ethanol overnight. Anesthesia was applied by an intraperitoneal injection of 0.8 ml of sodium pentobarbital. An area measuring 5 cm by 8 cm was shaved on the back of each rat. A total number of seven sutures were inserted in the rat’s dorsal skin aligned from the head to the tail as shown in Figure 6-2. Each suture sample was inserted from the upper right to the lower left by following a predetermined three-stitch suturing pattern as shown in the magnified image. A surgeon’s knot shown as an asterisk (*) was tied at the end of the barbed suture samples beforehand to act as a stopper for the suture deployment. The barbed anchoring section shown in blue was inserted into the subcutaneous layer following an identical suturing pattern as used in the in vitro suture/tissue pullout test described in Chapter 5. The non-barbed clamping section was also implanted under the skin to prevent biting and pulling by the rat. In order to maintain the desired tension on the unidirectional barbed sutures during the
experimental period, the barbed suture was further pulled to facilitate the engagement of the barbs with the surrounding tissue. A scale was used to check the tension (0.2 lb) applied on the suture. After the implantation of the barbed sutures, a disinfectant (benzalkonium bromide) was applied on the operative area.

Figure 6-2. Arrangement of suture samples in the rat dorsal skin showing the three-stitch suturing pattern

- Marker point
- Knot to maintain tension on the suture
- Suture without barbs (clamping section)
- Suture with barbs (anchoring section)

On the day of implantation, six three-stitch markers were drawn on the back of the rats. A number (1-6) generated in a random sequence was assigned to these six markers. The markers assigned an odd number were inserted with PDO barbed sutures, and the markers assigned an even number were inserted with P4HB barbed sutures. The top two suture samples were harvested for histological examination. One of the five rats in each group received non-barbed sutures at the top two marker locations to serve as controls for the histological examination. The other four markers
were inserted with barbed sutures and harvested for the biomechanical anchoring test. On the day of harvesting the skin tissue and immediately after euthanasia by an anesthesia overdose, a seventh marker was drawn and either suture type was inserted in the rat’s skin to represent the postoperative Day 0 control sample that was subsequently used for measuring the anchoring performance.

6.2.2 Methods of preventing loss of suture samples

Due to the discomfort and itchy feeling experienced after the operation, the rats tended to scratch, pull or bite the suture samples on their backs. In order to reduce the itchy feeling and the interference by rats, five strategies were used to prevent loss of suture samples.

1. Elizabethan collars (E-collars), as shown in Figure 6-3, were used to prevent the rat from biting and pulling on the inserted sutures. An initial trial of applying the E-collars to the rats was undertaken to check that the E-collars did not cause any redness or irritation around the neck, and did not restrict the rats ability to eat food and drink water. The E-collars were applied to the rats one day before the operation so the rats became accustomed to wearing them (Figure 6-3 (b)).

Figure 6-3. (a) Top view of the E-collar, (b) trial rat wearing the E-collar
2. A surgeon’s knot was tied at the end of the suture beforehand so as to maintain a tension on the suture sample and to prevent the barbed suture migrating in the direction of insertion. At the same time, the knots facilitated the daily inspection procedure to confirm the existence of the barbed sutures after the operation, as shown in Figure 6-4.

![Image](image-url)

Figure 6-4. Visible knots of the barbed sutures on the rat dorsal skin. The disinfectant has a pink color.

3. Each rat was kept and fed in an individual cage, which measured 40cm x 30cm x 20cm in size. This size ensured that the rat had freedom of movement with the E-collars.

4. In order to reduce the itchy feeling during the healing process, antiseptic and analgesic paenol, a phenolic herbal extract from the root of the peony tree, was applied on the first two post-operative days.

5. Each rat was inspected every day to check that the suture samples were still in place and that there were no signs of wound infection.

**6.2.3 Biomechanical anchoring test**

At each time point, fresh dorsal skin tissue containing the seven sutures was dissected from the rat. The skin tissue with the top two suture samples was preserved in 10% buffered formalin for histological examination. The rest of the skin tissue with the remaining five suture samples was harvested for the biomechanical anchoring test, as shown in Figure 6-5. In order to prevent
overstretching and distortion of the skin tissue while it was mounted on the customized U-shape clamps, the outline of the clamps was marked on the dorsal skin before tissue dissection. The non-barbed clamping section of each suture specimen was exposed by cutting and removing the fascial and adipose tissue from the subcutaneous side. The whole dorsal skin tissue sample was mounted between the flat clamps of a universal tensile tester. The clamping section of each suture specimen was clamped one at a time in the upper jaw, and the biomechanical anchoring test was performed at a crosshead speed of 20 mm/min in the direction opposition of the suture insertion direction. The excess tissue was cut after each test, and the upper jaw was lowered to clamp the next suture specimen. The maximum pullout force was recorded as the anchoring performance of the barbed suture.

Figure 6-5. Rat skin tissue with barbed suture samples mounted on the tensile tester using U-shape clamps

6.2.4 Histological analysis

At each time period of implantation, the fresh rat dorsal skin tissue containing the seven sutures was dissected from each sacrificed rat. The skin tissue with the top two suture samples was
cut into small pieces and preserved in 10% buffered formalin for histological examination. Dehydration was performed by running the tissues through a series of aqueous ethanol concentrations, namely: 60%, 70%, 80%, 95% and a second 95%. The tissues were embedded in glycol methacrylate (GMA) using a Technovit® 7100 embedding kit (Electron Microscopy Sciences, Hatfield, PA). Dehydration, infiltration, embedding and polymerization of the tissue samples were performed in the Histology Laboratory at NC State School of Veterinary Medicine. Detailed information about the embedding and sectioning procedures are described in the Appendix. Tissue blocks containing cross-sectional suture segments and longitudinal suture segments from the same rat were embedded in the same GMA resin block as shown in Figure 6-6. The blocks were rotated and aligned so that 4 μm thick sections could be cut to show the ideal position for viewing the longitudinal suture segments. The histological sections were then mounted and stained with hematoxylin-eosin stain.

The stained sections were examined semi-quantitatively by a trained veterinary pathologist, Dr. Debra Tokarz. The relative degree of tissue reaction for each suture specimen was assessed using a scoring scheme listed in Table 6-2 (Zaruby et al. 2011). A quantitative assessment of
cellular infiltration was taken within a circular view of 550 μm under 400x magnification. Four readings were taken in areas both around the barb and between barbs. Qualitative assessment of the other categories, namely, necrosis, congestion/edema, giant cells, fibrosis, neovascularity, calcification and fat infiltration, were recorded based on the extent visualized in the area of interest.

6.2.5 Statistical analysis

The effect of suture material and implantation time on the in vivo anchoring performance measured in the pullout test was analyzed by a two-way ANOVA using JMP Pro 13. Specific pairwise comparisons were made using least square means and Tukey-Kramer HSD posthoc analysis. Differences were considered significant at p < 0.05. The effect of suture material and implantation time on the histology results was analyzed by an ordinal logistic model at a significance level of p < 0.05.
Table 6-2. Tissue reaction scoring scheme by histologic categories (Zaruby et al. 2011)

<table>
<thead>
<tr>
<th>Histological category</th>
<th>Scoring system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular infiltrate</td>
<td>0-4†</td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
</tr>
<tr>
<td>Plasma cells</td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
</tr>
<tr>
<td>Degree of necrosis</td>
<td>0-4*</td>
</tr>
<tr>
<td>Congestion/edema</td>
<td></td>
</tr>
<tr>
<td>Giant cells</td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td></td>
</tr>
<tr>
<td>Neovascularity</td>
<td></td>
</tr>
<tr>
<td>Calcification</td>
<td></td>
</tr>
<tr>
<td>Fatty infiltration</td>
<td></td>
</tr>
<tr>
<td>Foreign body reaction</td>
<td>+/-!</td>
</tr>
</tbody>
</table>

† Number of cells scored from the average of four fields at x400 magnification:
0 = 0 cells, 1 = 1-5 cells, 2 = 6-15 cells, 3 = 16-25 cells, 4 = >25 cells.
* 0 = not present, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe
! Presence (+) of foreign body reaction related to the existence of giant cells

6.3 Results

6.3.1 Anchoring performance

The anchoring performance of barbed sutures measured as the maximum pullout force during the biomechanical anchoring test is shown in Figure 6-7. At Day 0 PDO barbed sutures had a significantly higher anchoring performance compared with P4HB, which corresponded to the in vitro pullout test results in the porcine model reported in Chapter 5. The anchoring performance of the PDO barbed sutures decreased continuously during the 28-day period of implantation. A
significant decrease in anchoring performance was observed for the P4HB barbed sutures during the first post-operative week. However, it then increased significantly between Day 14 and Day 28, which resulted in a significantly higher anchoring performance than for the PDO sutures. No significant difference in the anchoring performance was observed between the two materials on Day 14. For the failure mode, 90% of the PDO barbed sutures failed by suture breakage at Day 0, while the rest of PDO barbed sutures and all P4HB barbed sutures failed by barb slippage at all time points.

![Graph showing anchoring performance of PDO and P4HB barbed sutures](image)

Figure 6-7. Anchoring performance of PDO and P4HB barbed sutures as a function of implantation days (* PDO vs. P4HB p < 0.05)

6.3.2 Histological assessment

6.3.2.1 Semi-quantitative analysis of stained sections

Neutrophils, lymphocytes, plasma cells, macrophages and eosinophils are all white blood cells which participate in the inflammatory and immune responses to protect the body against both infectious disease and foreign material intruders. In the rat skin implanted with barbed sutures a robust inflammatory response was evident beginning with the earliest time point examined (Day 3).
The average of tissue reaction scores for both suture materials during the 28-day *in vivo* study are shown in Table 6-3. Since there was no significant difference between areas around the barb and areas between barbs, the histological scores were averaged into one average value. No significant difference was found in the scores between the barbed and non-barbed sutures. Therefore, the histological scores for the non-barbed sutures embedded in the fifth rat of each time point were excluded in the table. The quantitative analysis of infiltrated cells and the qualitative analysis of the other histological categories are plotted in Figures 6-8 and 6-9, respectively.
Table 6-3. Average tissue reaction scores for PDO and P4HB at Day 3, 7, 14 and Day 28

<table>
<thead>
<tr>
<th>Categories</th>
<th>day 3</th>
<th>day 7</th>
<th>day 14</th>
<th>day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDO</td>
<td>P4HB</td>
<td>PDO</td>
<td>P4HB</td>
</tr>
<tr>
<td>cellular infiltrate</td>
<td>3.78</td>
<td>3.61</td>
<td>3.94</td>
<td>3.69</td>
</tr>
<tr>
<td></td>
<td>3.69</td>
<td>3.94</td>
<td>3.69</td>
<td>3.91</td>
</tr>
<tr>
<td>neutrophils</td>
<td>1.91</td>
<td>1.43</td>
<td>2.16</td>
<td>1.97</td>
</tr>
<tr>
<td></td>
<td>0.91</td>
<td>2.69</td>
<td>1.56</td>
<td>1.50</td>
</tr>
<tr>
<td>lymphocytes</td>
<td>1.41</td>
<td>0.96</td>
<td>1.56</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>1.56</td>
<td>1.50</td>
<td>0.53</td>
<td>0.16</td>
</tr>
<tr>
<td>plasma cells</td>
<td>0.16</td>
<td>0.21</td>
<td>0.34</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>0.53</td>
<td>0.16</td>
<td>0.38</td>
<td>0.16</td>
</tr>
<tr>
<td>macrophages</td>
<td>3.56</td>
<td>3.29</td>
<td>3.84</td>
<td>3.22</td>
</tr>
<tr>
<td></td>
<td>3.22</td>
<td>3.22</td>
<td>3.66</td>
<td>3.59</td>
</tr>
<tr>
<td>eosinophils</td>
<td>0.47</td>
<td>0.25</td>
<td>0.22</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>0.22</td>
<td>0.66</td>
<td>0.84</td>
</tr>
</tbody>
</table>

| Degree of necrosis | 2.38 | 1.71 | 3.00 | 1.38 |
|                   | 1.75 | 1.50 | 0.63 | 0.50 |
| Congestion/Edema   | 0.88 | 1.71 | 1.13 | 0.63 |
| Giant cells        | 0.00 | 0.00 | 0.00 | 0.00 |
| Fibrosis           | 0.00 | 0.00 | 1.50 | 1.75 |
| Neovascularity     | 0.00 | 0.00 | 1.75 | 1.50 |
| Calcification      | 0.00 | 0.29 | 0.13 | 0.00 |
| Fatty infiltration | 0.00 | 0.00 | 0.00 | 0.00 |
| Foreign body reaction | - | - | + | - |

Cellular infiltration was scored based on the total cell number in the view of interest. During the 28-day implantation period, large numbers of cells infiltrated into the operative area. No significant difference of cellular infiltration was found between the two materials nor between different time points. However, when looking at the specific cellular response, the PDO barbed sutures had significantly more infiltrated lymphocytes, plasma cells, macrophages and eosinophils than the P4HB barbed sutures. Macrophages had the largest number for both materials during the whole implantation period. The amount of neutrophils and lymphocytes were similar, however, more lymphocytes infiltrated around the PDO barbed sutures while more neutrophils infiltrated around the P4HB barbed sutures. Minimal plasma cells and eosinophils were observed.
The mild to moderate degrees of necrosis were observed to decrease as the implantation time increased. The degree of necrosis of PDO barbed sutures was higher than that of P4HB barbed sutures at all four time points, and at Day 7, a significant difference was observed. The degree of congestion/edema was minimal for both suture materials, and in fact at Day 28 there was no evidence of edema for the PDO barbed sutures. Foreign body giant cells were observed only at Days 14 and 28, with a significantly higher number for the PDO barbed sutures. Evidence of mild fibrosis was seen in both suture materials with no significant differences, and only minimal levels of neovascularity and calcification were observed for both the PDO and P4HB barbed sutures. No fatty infiltration was observed for either suture material during the 28-day implantation study.
6.3.2.2 Histological interactions between barbs and the surrounding tissues

The anchoring performance of barbed sutures is achieved by opening the barbs so they penetrate and engage the surrounding tissues. The extent of the anchoring ability can be measured by the two test methods described in Chapter 5 or by an assessment of the outcome and function of the sutured tissues. The physical interaction of barbs with the surrounding tissue has not previously been studied. In addition to the histological scores examined on the stained sections, the cross-sectional histopathological views are listed in Table 6-4. They explain how the longitudinal views reveal the way the barbs behave while anchoring the surrounding tissue. Due to the large difference in hardness between the suture material and the GMA resin, the suture material often fell out of the section, and was missing in most cross-sectional sections. Crumpled tissue sections were observed more in the longitudinal direction due to the contraction of the tissue slice anchored with the barbs that did not spread well in the water because of the hydrophobicity.
The ideal position for the cross-sectional section and the longitudinal section would be similar to PDO at Day 3 and P4HB Day 28. However, it was difficult to see the suture material through the tissue block and align the suture to the ideal longitudinal position. There was a significant chance of cutting the non-barbed section in the cross-sectional direction when the ideal longitudinal view was achieved.

The anchoring performance of barbed sutures was first visualized by the tissues pinched into the suture underneath the barbs in a combined view of both the cross-sectional and longitudinal sections. Some barbs were open wide and intimately engaged with the surrounding tissue, while some barbs were barely open with no tissue engagement. Since the barbed sutures were not pulled out, few barbs had failed in a manner of bending or peeling. However, barb breakage from the suture monofilament was observed among the PDO sutures at Day 28.
Table 6-4. Histopathological views of the barbed sutures in rat skin tissue

<table>
<thead>
<tr>
<th>Day #</th>
<th>PDO</th>
<th>P4HB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cross-sectional</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>3</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
</tr>
<tr>
<td>7</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
</tr>
<tr>
<td>14</td>
<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
</tr>
<tr>
<td>28</td>
<td><img src="image13" alt="Image" /></td>
<td><img src="image14" alt="Image" /></td>
</tr>
</tbody>
</table>
Histopathological images of the P4HB barbed sutures are shown in Figure 6-10 at four different time points during the *in vivo* study. Histological sections at Day 3 show an acute inflammatory response with large numbers of white blood cells migrated into the implantation zone which is predominantly necrotic, congested and edematous. Sections from Day 7 onwards began to exhibit fibroplasia with the presence of neovascularization and active fibroblasts, but with limited collagen deposition. At Days 14 and 28, fibroplasia was still present, but the deposition of collagen matrix was now more evident. Maturing fibrous tissue with long thin fibroblasts embedded in a pink collagen matrix were observed at Day 28 and highlighted within the yellow zone in Figure 6-10 (d). Barbed suture material was encapsulated by fibroplasia or fibrosis around the circumference of the suture as shown in Figure 6-11. The thickness of the capsule formed around the PDO barbed sutures was thicker than that around the P4HB barbed sutures. Suture material resorption was clearly evident for the PDO barbed sutures at Day 28, while no resorption was noticeable for the P4HB barbed sutures at Day 28.
Figure 6-10. Scored items of barbed sutures marked in histopathological images, (a) PDO at Day 3 under 400X, (b) P4HB at Day 7 under 200X, (c) PDO at Day 14 under 400X and (d) P4HB at Day 28 under 200X

Figure 6-11. Histopathological images of encapsulation and material absorption at Day 28 under 400X, (a) PDO and (b) P4HB
6.4 Discussions

PDO barbed sutures had a significantly higher anchoring performance in rat skin tissue at Day 0, which was consistent with the results found previously in porcine skin. The flexibility and low bending stiffness of the barbs on P4HB monofilaments may have had difficulty in anchoring the surrounding tissue during the immediate period following implantation. However, the maximum pullout force for both suture materials measured in rat skin was lower than that measured in porcine skin. This might be attributed to the difference in animal species and the different thicknesses of the dermal and epidermal layers. The rat skin tissue was much thinner than the porcine skin tissue, and the barbed sutures were more engaged in the deeper subdermal and subcutaneous adipose tissues rather than in the denser epidermis and dermis. This was confirmed by the histological sections. In fact the effect of species variability on the anchoring performance of barbed sutures to close wounds has been observed previously in a canine versus a porcine model (Law et al. 2017; Zaruby et al. 2011).

The anchoring performance of both PDO and P4HB suture materials decreased at Day 3 and Day 7. This loss in anchoring strength was believed to be due to the removal of collagen and extracellular matrix by neutrophils and macrophages during the inflammatory phase of wound healing (Sheikh et al., 2015). Another reason for the reduced anchoring performance might have been due to suture movement or displacement of the suture during scratching of the back or rubbing and abrasion against the cage. Even though one end of the implanted suture was fixed with a surgeon’s knot, the barbed sutures with only 20 barbs was able to move within the flexible skin tissue, and may have resulted in barbed suture damage prior to the suture/tissue pullout test. An increase of the anchoring performance was observed for the P4HB barbed suture at Day 14 and Day 28, while that of the PDO barbed sutures continued to decrease. By the end of the 28-
day implantation period, the P4HB barbed sutures had a significantly higher anchoring performance than the PDO. This could be attributed to the deposition of collagen matrix and the formation of a tissue capsule, which helped the barbs anchor and engage with surrounding tissue. The fragmentation of the PDO suture material was evident at Day 28 due to its rapid degradation rate, which could have resulted in barb breakage and loss of anchoring. In addition to the hydrolytic degradation, the presence of giant cells around the operative area accelerated the fragmentation of the suture material. The absorption of PDO sutures in the current study appeared to be much earlier than the 90 days reported in Ray’s study (Ray et al., 1981). This may have been due to the overnight sterilization of the suture samples in 75% ethanol, which was much longer than the conditions reported by Shuttleworth et al. who have shown that size 8-0 Vicryl® (polyglactin 910) can be sterilized by 20 min immersion in 70% isopropanol without affecting the mechanical properties (Shuttleworth, et al., 1999).

The overall tissue reaction score for P4HB barbed sutures was lower than that of PDO barbed sutures. MonoMax® suture has been demonstrated to have good biocompatibility by a number of different in vitro and in vivo test methods (Odermatt et al. 2012). The slow degradation rate has helped to reduce the release of small acid molecules that could interfere the pH value at the implantation site (Williams, et al., 2013). The flexibility and low bending stiffness of the P4HB barbs may provoke less irritation than the stiffer PDO barbs, even though the P4HB barbs are associated with an inferior anchoring performance at early implantation times (Odermatt et al. 2012).

There have been a variety of results for the histological assessment of barbed sutures reported by different studies. These variations might be attributed to differences in several factors such as the suture material, the animal species, the anatomical location, the implantation time,
suturing technique and scoring scheme. Due to the limitation of available barbed suture materials, many comparison studies have been conducted on different suture materials. Demyttenaere et al. compared the healing of barbed and non-barbed Maxon sutures for gastrointestinal closure and found no differences on inflammation, histological alignment or adhesion (Demyttenaere et al. 2009). Animal models involved with histological studies of barbed sutures include the rat, pig, dog and ewe (female sheep). Differences in the extent and severity of their physiological symptoms are important factors. The scoring scheme for histological assessment used in the current study was developed by Zaruby et al. and Law et al. (Zaruby et al. 2011; Law et al. 2017). In order to compare the histological results, the average score was calculated for cell infiltration, necrosis, congestion/edema, foreign body giant cells, fibrosis and neovascularization. The average score for tissue reaction was slightly higher for the V-Loc™ barbed sutures in a canine model due to intense cellular infiltration. Whereas the mildest inflammatory reaction was observed with V-Loc™ and Quill Monoderm™ barbed sutures in a porcine model. This was most likely due to the administration of anti-inflammatory drugs and an average score calculated using different histological categories. In addition to wound closure of the skin, which is relatively straightforward with easy handling, barbed sutures have been widely used in the repair of other organs where additional functions or requirements are needed. For example, V-Loc™ and Vicryl were compared during two studies involving the closure of the bladder wall and uterine horns (Petrut et al. 2013; Api et al. 2015). Lower scores for tissue reaction were observed for bladder wall closure with V-Loc™ barbed sutures, while significantly higher inflammatory cell scores were generated following uterine horn closure. One similarity in both of these studies was that more tissue adhesions were observed in the barbed suture group, which may be due to the suturing technique used. Closure for most organs inside the abdominal cavity uses a continuous running unlocked
suturing technique (Api et al. 2015; Demyttenaere et al. 2009; Petrut et al. 2013). So the suture material including the barbs would have been exposed to both the organ tissue and the surface of the abdominal wall. So in the event of organ movement, the barbs might have provoked the formation of tissue adhesions. For the closure of skin tissue, barbed sutures are normally buried inside the dermal layer using a sinusoidal wave suturing technique similar to the wound closure test method described in Chapter 5. Note that whenever a closed wound is sutured under tension, any movement of the suture barbs can trigger a more severe inflammatory response. The scoring scheme for histological assessment is always of critical importance whenever the results are compared. The tissue reaction can either be assessed quantitatively, based on the cell number and the thickness or area of the capsule, or it can be assessed qualitatively, based on the extent of specific symptoms. Lastly, the duration of implantation, which may have been several days, weeks or even several months would have affected the histological assessment.

The present study is the first to compare PDO and P4HB barbed sutures using a rat model, and to visualize the barb-tissue interactions in both cross-sectional and longitudinal histological sections. However, the 28-day implantation study may not have been long enough to reveal all the advantages of using P4HB because of its long-term degradation profile. The maximum pullout force measured by means of a suture/tissue pullout test is likely to have underrated the anchoring performance in an actual wound closure situation.

6.5 Chapter summary

The in vivo anchoring performance of PDO and P4HB barbed sutures have been successfully measured in a suture/tissue pullout test using a rat model. The maximum pullout force for the PDO barbed sutures decreased continuously over the 28-day implantation time, while that
of P4HB barbed sutures increased between Days 14 and 28. This study is the first to directly
visualize the histological interaction between suture barbs and the surrounding tissue using a
combined cross-sectional and longitudinal sectional viewing procedure. In general, the P4HB
barbed sutures generated a less severe inflammatory response than PDO barbed sutures with lower
degree of necrosis and giant cells observed in the rat skin tissue.
CHAPTER 7: CONCLUSIONS, LIMITATIONS AND FUTURE WORK

7.1 Conclusions

The new generation of bacterial polyester, P4HB monofilament, has been compared with a well-studied absorbable suture material, PDO, in order to determine its suitability for use in barbed suture applications. To address this main goal, five specific objectives were identified and are described in greater detail in Chapter 1, Section 1.2. Here in Chapter 7 we revisit these five objectives in order to evaluate whether or not each one has been achieved. The following section describes the conclusions associated with each objective, and, if the objective has not been achieved then an explanation of why the methodological approach has not worked.

The first objective was to characterize the material properties of the two suture materials using a range of different analytical techniques. This included the mechanical, thermal and structural properties, of P4HB and PDO suture materials. Now that these tests have been completed, it has been found that P4HB has a significantly higher tensile strength and stiffness compared to PDO, while at the same time being softer, which means having a significantly lower hardness value. PDO has a significantly higher melting point and crystalline structure orientation.

The second objective involved the fabrication of barbed sutures from PDO and P4HB monofilaments and to compare their barb geometry and mechanical properties with two commercial barbed sutures already on the market. Size 0, 2-0 and 3-0 barbed sutures with 120° staggered barbs were fabricated from PDO and P4HB monofilaments using a manual mechanical barbing process. P4HB barbed sutures had equivalent tensile strength to the PDO barbed sutures of the same size, as well as two equivalent commercial barbed surgical sutures called Quill™ PDO and Monoderm™. A marginally deeper cut depth was achieved for the experimentally barbed P4HB sutures due to its greater softness compared to PDO.
The third objective involved exposing PDO and P4HB barbed and non-barbed sutures to a 10-week hydrolytic degradation study, and monitoring the changes in mechanical, thermal and surface properties during this period of controlled degradation. The rapid degradation rate of PDO resulted in a quick loss of mechanical stability and morphological integrity during the 10 weeks of hydrolysis. In comparison, P4HB had more resistance to hydrolysis which is attributed to its higher molecular weight. The increased surface area created by the barbing process had no significant influence on the degradation rate, and the geometric integrity of the barbs for both suture materials was maintained over the ten weeks of degradation.

The fourth objective involved measuring the *in vitro* anchoring performance of PDO and P4HB barbed sutures in porcine skin tissue. Two methods were used to monitor the anchoring performance, including both a suture/tissue pullout test as well as a wound closure test. The type of suture material, the suture size, the barb geometry, the type and shape of the needle as well as the needle point were all found to have a significant influence on the anchoring performance measured as the maximum pullout force. However, the anchoring performance measured by the wound closure test method was more related to the suture material rather than the suture size. No correlation was found between the anchoring performances measured by these two test methods. An explanation of this phenomenon needs additional study, but it is believed that the lack of correlation is due in part to the different failure modes that were observed.

In the final and fifth objective, the *in vivo* anchoring performances of PDO and P4HB barbed sutures were compared by measuring the maximum pullout force in skin using a 4-week rat study. PDO barbed sutures showed a significantly higher anchoring performance in the first week thanks to a higher stiffness and surface hardness. However, the anchoring behavior fell continuously with time, due to a rapid degradation rate and phagocytosis of foreign body giant
cells. The anchoring performance of the P4HB barbed sutures increased between Days 14 and 28 and, by the end of the study, achieved a significantly higher anchoring performance in comparison to the PDO barbed sutures. In general, P4HB barbed sutures generated a less severe inflammatory response than PDO when implanted in the rat’s dorsal dermal tissue. By combining the views from the cross-sectional and longitudinal sections we were able to directly present for the first time the histological interactions between the barbs and the surrounding tissue. This was due to the fact that with this sectioning approach a direct view of the whole barb configuration and the availability of assessing tissue anchoring underneath the barb was now possible.

Although not currently used as a knotless wound closure device, laboratory fabricated P4HB barbed sutures used in the current study have shown equivalent suture strength, in vitro anchoring performance and a significantly higher in vivo anchoring performance compared with the use of PDO barbed sutures. In summary, P4HB is a promising candidate as a barbed suture material, especially for long-term applications.

7.2 Limitations

A number of properties have been measured and compared between PDO and P4HB monofilaments. However, due to the different synthesis methods and the lack of knowledge on manufacturing conditions such as the draw ratio, some mismatched results (e.g., P4HB had a higher stiffness but a lower hardness and a lower indentation elastic modulus) were hard to explain without assumptions. For the microstructure, the crystallinity of P4HB is not achievable due to the lack of studies on the 100% crystalline and amorphous structures. P4HB monofilaments have been measured to have a significant higher tensile strength than PDO. However, the loss of suture strength was significantly higher than for the PDO barbed sutures due to a deeper cut depth. The
mechanical barbing machine used in this study was a manual prototype model, which was not able to achieve the desired cut depth target for P4HB. The degradation study exposed the suture samples only to pH-driven hydrolytic conditions under no tension. The change of structural integrity of barbs may be different in the situation of an enzymatic hydrolysis with tension applied. The anchoring performance measured in both porcine and rat skin tissues was a demonstration of the potential of P4HB being used as an absorbable barbed suture material. Further studies are necessary to predict its performance in clinical procedures.

7.3 Future work

To have an in-depth study of the relationship between the suture strength loss and the effective cross-sectional area remaining, one could fabricate barbed suture samples with different sets (one, two and three) of cuts aligned with 120° staggered spacing and assess the change in strength relative to the change in cross-sectional area. To explore the change in anchoring performance in a controlled degradation condition, one could measure the maximum pullout force of barbed sutures after the desired degradation time. To obtain a realistic prediction of clinical suturing techniques, one could measure the in vivo anchoring performance of P4HB barbed sutures with a more realistic suturing technique. Additionally, to maximize the engagement between the barbs and the surrounding tissue with minimal loss in suture strength, one could optimize the barb geometry by measuring the tensile strength and the anchoring performance of barbed sutures with different combinations of cut angle and cut depth. Furthermore, researchers with expertise in polymer chemistry and physics could tune the polymer and fiber properties of P4HB monofilaments by applying a higher draw ratio to achieve a higher degree of crystallinity, higher strength and superior surface hardness.
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Appendix A

All data in the tables are presented as mean (standard deviation).

A. Diameter measurement of PDO and P4HB monofilaments (n = 10, Section 3.1)

<table>
<thead>
<tr>
<th>Suture size</th>
<th>Size 0</th>
<th>Size 2-0</th>
<th>Size 3-0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>PDO</td>
<td>P4HB</td>
<td>PDO</td>
</tr>
<tr>
<td>Diameter (µm)</td>
<td>485.4 (3.4)</td>
<td>450.4 (3.0)</td>
<td>376.6 (7.1)</td>
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B. Tensile properties of PDO and P4HB monofilaments (n = 5, Section 3.1.1)

<table>
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<th>Suture size</th>
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<th>Size 2-0</th>
<th>Size 3-0</th>
</tr>
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<tbody>
<tr>
<td>Material</td>
<td>PDO</td>
<td>P4HB</td>
<td>PDO</td>
</tr>
<tr>
<td>Max. tensile force (N)</td>
<td>80.92 (0.44)</td>
<td>136.63 (3.08)</td>
<td>43.19 (3.10)</td>
</tr>
<tr>
<td>Elongation at break (mm)</td>
<td>164.29 (8.89)</td>
<td>92.80 (1.71)</td>
<td>132.10 (8.27)</td>
</tr>
<tr>
<td>Stiffness (N/mm)</td>
<td>0.609 (0.032)</td>
<td>0.884 (0.011)</td>
<td>0.358 (0.023)</td>
</tr>
<tr>
<td>Work to rupture (J)</td>
<td>6.81 (0.59)</td>
<td>4.98 (0.24)</td>
<td>2.67 (0.35)</td>
</tr>
</tbody>
</table>
C. Nanoindentation results of size 2-0 PDO and P4HB monofilaments (n = 3, Section 3.1.3)

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<thead>
<tr>
<th>Distance from edge (µm)</th>
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<th>105</th>
<th>180</th>
<th>255</th>
<th>330</th>
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</thead>
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<tr>
<td><strong>Hardness (MPa)</strong></td>
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<td></td>
<td></td>
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<tr>
<td>PDO-C</td>
<td>148.89</td>
<td>128.51</td>
<td>154.41</td>
<td>126.50</td>
<td>171.79</td>
</tr>
<tr>
<td>PDO-L</td>
<td>157.40</td>
<td>138.67</td>
<td>107.25</td>
<td>158</td>
<td>147.86</td>
</tr>
<tr>
<td>P4HB-C</td>
<td>81.94</td>
<td>56.66</td>
<td>64.31</td>
<td>73.46</td>
<td>55.31</td>
</tr>
<tr>
<td>P4HB-L</td>
<td>88.15</td>
<td>98.27</td>
<td>87.21</td>
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<td>88.72</td>
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<tr>
<td><strong>Modulus (GPa)</strong></td>
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<td></td>
<td></td>
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<tr>
<td>PDO-C</td>
<td>1.85</td>
<td>1.85</td>
<td>1.97</td>
<td>2.06</td>
<td>2.36</td>
</tr>
<tr>
<td>PDO-L</td>
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<td>2.29</td>
<td>3.14</td>
<td>3.86</td>
<td>2.81</td>
</tr>
<tr>
<td>P4HB-C</td>
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<td>0.72</td>
<td>0.76</td>
<td>0.81</td>
<td>0.72</td>
</tr>
<tr>
<td>P4HB-L</td>
<td>0.86</td>
<td>0.94</td>
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<td>0.85</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>Creep (%)</strong></td>
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<td></td>
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<td>PDO-C</td>
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<td>PDO-L</td>
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<td>2.44</td>
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C: cross-sectional section; L: longitudinal section
D. Results of barb geometry (n = 10, Section 3.2)

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<td>P4HB</td>
<td>PDO</td>
</tr>
<tr>
<td>Cut angle (°)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>162.6 (1.7)</td>
<td>165.6 (1.3)</td>
<td>164.8 (1.9)</td>
</tr>
<tr>
<td>Cut depth (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.9 (1.1)</td>
<td>31.2 (3.0)</td>
<td>31.2 (5.5)</td>
</tr>
<tr>
<td>Barb length (µm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>350.9 (19.6)</td>
<td>558.8 (18.5)</td>
<td>440.2 (42.5)</td>
</tr>
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</table>

E. Tensile properties of barbed PDO and P4HB sutures (n = 5, Section 3.2)

<table>
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<th>3-0</th>
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<tbody>
<tr>
<td>Material</td>
<td>PDO</td>
<td>P4HB</td>
<td>PDO</td>
</tr>
<tr>
<td>Max. tensile force (N)</td>
<td>45.51 (3.29)</td>
<td>48.52 (7.23)</td>
<td>24.42 (1.28)</td>
</tr>
<tr>
<td>Elongation at break (mm)</td>
<td>83.28 (4.64)</td>
<td>54.34 (5.32)</td>
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<td>Stiffness (N/m)</td>
<td>576.8 (25.3)</td>
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<td>Work to rupture (J)</td>
<td>1.84 (0.26)</td>
<td>1.13 (0.25)</td>
<td>1.03 (0.10)</td>
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</table>
F. Weight retention (%) of suture samples during hydrolytic degradation (n = 5, Section 4.3.2)

<table>
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<tr>
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<th>PDO-B</th>
<th>P4HB-N</th>
<th>P4HB-B</th>
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<td>(0.2)</td>
<td>(0.3)</td>
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<td>(0.2)</td>
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N: non-barbed; B: barbed

G. Diameter (μm) of suture samples during hydrolytic degradation (n = 5, Section 4.3.2)

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<th>PDO</th>
<th>PDO</th>
<th>PDO</th>
<th>P4HB</th>
<th>P4HB</th>
<th>P4HB</th>
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<tr>
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H. Maximum tensile force (N) of suture samples during hydrolytic degradation (n = 5, Section 4.3.3.1)

<table>
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<tr>
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<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>PDO-N</td>
<td>52.21</td>
<td>46.85</td>
<td>42.33</td>
<td>37.06</td>
<td>28.93</td>
<td>20.29</td>
</tr>
<tr>
<td></td>
<td>(2.89)</td>
<td>(3.37)</td>
<td>(3.93)</td>
<td>(1.92)</td>
<td>(5.49)</td>
<td>(2.82)</td>
</tr>
<tr>
<td>PDO-B</td>
<td>30.31</td>
<td>25.97</td>
<td>22.26</td>
<td>17.79</td>
<td>10.06</td>
<td>6.79</td>
</tr>
<tr>
<td></td>
<td>(1.58)</td>
<td>(1.54)</td>
<td>(2.60)</td>
<td>(1.45)</td>
<td>(1.83)</td>
<td>(1.03)</td>
</tr>
<tr>
<td>P4HB-N</td>
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<td>(1.98)</td>
<td>(2.69)</td>
<td>(3.25)</td>
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<td>(3.04)</td>
<td>(2.51)</td>
<td>(2.01)</td>
<td>(1.65)</td>
<td>(2.34)</td>
<td>(2.23)</td>
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</tbody>
</table>

<table>
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<th>7</th>
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<th>9</th>
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<tr>
<td>PDO-N</td>
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<td>3.51</td>
<td>3.34</td>
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<td></td>
<td>(1.31)</td>
<td>(0.46)</td>
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<td>(2.27)</td>
<td>(2.57)</td>
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<td>(3.81)</td>
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I. Strength retention (%) of suture samples during hydrolytic degradation \( (n = 5, \text{Section 4.3.3.1}) \)

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<tr>
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<td>24.7</td>
<td>15.1</td>
<td>9.6</td>
<td>5.4</td>
<td>5.1</td>
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<tr>
<td>PDO-B</td>
<td>14.3</td>
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<td>P4HB-N</td>
<td>82.5</td>
<td>75.6</td>
<td>74.1</td>
<td>89.7</td>
<td>82.0</td>
</tr>
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<td>P4HB-B</td>
<td>76.5</td>
<td>59.6</td>
<td>77.2</td>
<td>96.2</td>
<td>89.2</td>
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</table>

J. Strain at break (%) of suture samples during hydrolytic degradation \( (n = 5, \text{Section 4.3.3.2}) \)

<table>
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<td>60.6</td>
<td>68.0</td>
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<td>48.6</td>
<td>36.8</td>
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<tr>
<td>PDO-B</td>
<td>37.0</td>
<td>39.3</td>
<td>35.5</td>
<td>23.5</td>
<td>12.2</td>
<td>7.2</td>
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<tr>
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<td>55.6</td>
<td>65.0</td>
<td>63.4</td>
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<td>64.6</td>
</tr>
<tr>
<td>P4HB-B</td>
<td>18.3</td>
<td>24.2</td>
<td>18.9</td>
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<td>4.2</td>
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<td>PDO-B</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>P4HB-N</td>
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<td>66.7</td>
<td>68.5</td>
<td>63.7</td>
<td>64.6</td>
</tr>
<tr>
<td>P4HB-B</td>
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<td>30.7</td>
<td>31.6</td>
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</table>
K. Stiffness (N/mm) of suture samples during hydrolytic degradation (n = 5, Section 4.3.3.3)

<table>
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<tbody>
<tr>
<td>PDO-N</td>
<td>0.427</td>
<td>0.329</td>
<td>0.330</td>
<td>0.391</td>
<td>0.453</td>
<td>0.511</td>
</tr>
<tr>
<td></td>
<td>(0.006)</td>
<td>(0.006)</td>
<td>(0.004)</td>
<td>(0.008)</td>
<td>(0.019)</td>
<td>(0.015)</td>
</tr>
<tr>
<td>PDO-B</td>
<td>0.404</td>
<td>0.320</td>
<td>0.329</td>
<td>0.382</td>
<td>0.427</td>
<td>0.510</td>
</tr>
<tr>
<td></td>
<td>(0.033)</td>
<td>(0.004)</td>
<td>(0.022)</td>
<td>(0.013)</td>
<td>(0.006)</td>
<td>(0.007)</td>
</tr>
<tr>
<td>P4HB-N</td>
<td>0.509</td>
<td>0.403</td>
<td>0.387</td>
<td>0.414</td>
<td>0.320</td>
<td>0.376</td>
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<tr>
<td></td>
<td>(0.005)</td>
<td>(0.034)</td>
<td>(0.016)</td>
<td>(0.022)</td>
<td>(0.052)</td>
<td>(0.026)</td>
</tr>
<tr>
<td>P4HB-B</td>
<td>0.809</td>
<td>0.569</td>
<td>0.802</td>
<td>0.779</td>
<td>0.355</td>
<td>0.448</td>
</tr>
<tr>
<td></td>
<td>(0.015)</td>
<td>(0.024)</td>
<td>(0.019)</td>
<td>(0.063)</td>
<td>(0.025)</td>
<td>(0.031)</td>
</tr>
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</table>

<table>
<thead>
<tr>
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</thead>
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<td>PDO-N</td>
<td>525.8</td>
<td>588.0</td>
<td>623.6</td>
<td>738.5</td>
<td>717.4</td>
</tr>
<tr>
<td></td>
<td>(19.6)</td>
<td>(59.9)</td>
<td>(4.2)</td>
<td>(70.7)</td>
<td>(56.8)</td>
</tr>
<tr>
<td>PDO-B</td>
<td>523.3</td>
<td>574.8</td>
<td>619.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(38.5)</td>
<td>(14.1)</td>
<td>(17.6)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P4HB-N</td>
<td>374.8</td>
<td>315.7</td>
<td>339.1</td>
<td>358.8</td>
<td>407.4</td>
</tr>
<tr>
<td></td>
<td>(3.27)</td>
<td>(47.3)</td>
<td>(92.5)</td>
<td>(109.0)</td>
<td>(17.0)</td>
</tr>
<tr>
<td>P4HB-B</td>
<td>422.7</td>
<td>371.2</td>
<td>440.1</td>
<td>503.4</td>
<td>444.4</td>
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<tr>
<td></td>
<td>(23.4)</td>
<td>(38.8)</td>
<td>(30.6)</td>
<td>(20.5)</td>
<td>(20.1)</td>
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L. Toughness (J) of suture samples during hydrolytic degradation (n = 5, Section 4.3.3.4)

<table>
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<tbody>
<tr>
<td>PDO-N</td>
<td>3.246</td>
<td>3.205</td>
<td>2.641</td>
<td>1.872</td>
<td>1.170</td>
<td>0.552</td>
</tr>
<tr>
<td></td>
<td>(0.409)</td>
<td>(0.497)</td>
<td>(0.553)</td>
<td>(0.225)</td>
<td>(0.428)</td>
<td>(0.164)</td>
</tr>
<tr>
<td>PDO-B</td>
<td>1.142</td>
<td>1.025</td>
<td>0.887</td>
<td>0.434</td>
<td>0.129</td>
<td>0.049</td>
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<tr>
<td></td>
<td>(0.071)</td>
<td>(0.129)</td>
<td>(0.178)</td>
<td>(0.076)</td>
<td>(0.054)</td>
<td>(0.020)</td>
</tr>
<tr>
<td>P4HB-N</td>
<td>4.528</td>
<td>5.052</td>
<td>4.661</td>
<td>4.714</td>
<td>4.050</td>
<td>4.264</td>
</tr>
<tr>
<td></td>
<td>(0.380)</td>
<td>(0.410)</td>
<td>(0.400)</td>
<td>(0.470)</td>
<td>(0.460)</td>
<td>(0.600)</td>
</tr>
<tr>
<td>P4HB-B</td>
<td>0.520</td>
<td>0.630</td>
<td>0.518</td>
<td>0.495</td>
<td>0.562</td>
<td>0.676</td>
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<td></td>
<td>(0.086)</td>
<td>(0.106)</td>
<td>(0.055)</td>
<td>(0.027)</td>
<td>(0.091)</td>
<td>(0.089)</td>
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</table>

<table>
<thead>
<tr>
<th>Weeks</th>
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<tr>
<td>PDO-N</td>
<td>0.217</td>
<td>0.058</td>
<td>0.021</td>
<td>0.003</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(0.12)</td>
<td>(0.019)</td>
<td>(0.000)</td>
<td>(0.000)</td>
<td>-</td>
</tr>
<tr>
<td>PDO-B</td>
<td>0.017</td>
<td>0.007</td>
<td>0.006</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(0.012)</td>
<td>(0.002)</td>
<td>(0.002)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P4HB-N</td>
<td>4.886</td>
<td>4.307</td>
<td>4.469</td>
<td>5.112</td>
<td>4.668</td>
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<td></td>
<td>(0.345)</td>
<td>(0.904)</td>
<td>(0.824)</td>
<td>(0.438)</td>
<td>(0.575)</td>
</tr>
<tr>
<td>P4HB-B</td>
<td>0.634</td>
<td>0.442</td>
<td>0.629</td>
<td>0.824</td>
<td>0.775</td>
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<td>(0.097)</td>
<td>(0.082)</td>
<td>(0.089)</td>
<td>(0.184)</td>
<td>(0.087)</td>
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L. Thermal properties of suture samples during hydrolytic degradation (n = 2, data of one replicate is listed in the table, Section 4.3.4)

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<tbody>
<tr>
<td>Melting temperature (°C)</td>
<td>PDO</td>
<td>99.8</td>
<td>101.1</td>
<td>102.2</td>
<td>103.2</td>
<td>104.9</td>
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<tr>
<td></td>
<td>P4HB</td>
<td>60.9</td>
<td>65.1</td>
<td>65.9</td>
<td>66.8</td>
<td>66.7</td>
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<tr>
<td>Heat of fusion (J/g)</td>
<td>PDO</td>
<td>80.11</td>
<td>82.84</td>
<td>84.81</td>
<td>85.43</td>
<td>87.89</td>
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<tr>
<td></td>
<td>P4HB</td>
<td>63.46</td>
<td>65.58</td>
<td>67.56</td>
<td>66.44</td>
<td>62.34</td>
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<tr>
<td>Crystallinity (%)</td>
<td>PDO</td>
<td>57.9</td>
<td>57.8</td>
<td>60.2</td>
<td>60.7</td>
<td>62.4</td>
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<table>
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<tbody>
<tr>
<td>Melting temperature (°C)</td>
<td>PDO</td>
<td>105.7</td>
<td>106.0</td>
<td>106.6</td>
<td>106.5</td>
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<tr>
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<td>P4HB</td>
<td>67.6</td>
<td>68.3</td>
<td>68.8</td>
<td>68.6</td>
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<tr>
<td>Heat of fusion (J/g)</td>
<td>PDO</td>
<td>89.38</td>
<td>92.78</td>
<td>91.01</td>
<td>96.65</td>
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<tr>
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<td>P4HB</td>
<td>63.84</td>
<td>66.12</td>
<td>67.50</td>
<td>64.15</td>
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<tr>
<td>Crystallinity (%)</td>
<td>PDO</td>
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<td>65.9</td>
<td>64.6</td>
<td>68.6</td>
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L. GPC results of P4HB during hydrolytic degradation (n = 1, Section 4.3.5)

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<tr>
<td>Molecular weight (Dalton * 10^3)</td>
<td>31.55</td>
<td>30.98</td>
<td>30.18</td>
<td>29.59</td>
<td>29.93</td>
<td>28.69</td>
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<tr>
<td>PDI</td>
<td>2.12</td>
<td>2.00</td>
<td>2.08</td>
<td>2.06</td>
<td>1.95</td>
<td>2.10</td>
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<table>
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<th>Weeks</th>
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<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
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<td>Melting temperature (°C)</td>
<td>26.95</td>
<td>26.77</td>
<td>24.69</td>
<td>27.06</td>
<td>25.67</td>
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<tr>
<td>PDI</td>
<td>2.05</td>
<td>1.97</td>
<td>2.26</td>
<td>2.23</td>
<td>1.93</td>
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M. *In vitro* anchoring performance of barbed sutures measured in the Full Factorial Design I (n = 6, Section 5.3.1)

<table>
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<tr>
<th>Suture material</th>
<th>PDO</th>
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<tbody>
<tr>
<td>Barb length</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suture size</td>
<td>0</td>
<td>2-0</td>
<td>3-0</td>
</tr>
<tr>
<td>Maximum pullout force (N)</td>
<td>13.59</td>
<td>11.09</td>
<td>9.27</td>
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<table>
<thead>
<tr>
<th></th>
<th>Short barb</th>
<th>Long barb</th>
</tr>
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<tbody>
<tr>
<td>PDO</td>
<td>(1.21)</td>
<td>(1.66)</td>
</tr>
<tr>
<td>P4HB</td>
<td>(1.72)</td>
<td>(0.86)</td>
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</table>

N. *In vitro* anchoring performance of barbed sutures measured in the Full Factorial Design II (n = 6, Section 5.3.1)

<table>
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<th>Suture material</th>
<th>PDO</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Needle type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue type</td>
<td>Epidermis</td>
<td>Dermis</td>
<td>Epidermis</td>
</tr>
<tr>
<td>Maximum pullout force (N)</td>
<td>14.58 (1.49)</td>
<td>11.47 (1.72)</td>
<td>10.60 (0.70)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Taper point</th>
<th>Diamond point</th>
</tr>
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<tbody>
<tr>
<td>PDO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4HB</td>
<td></td>
<td></td>
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</tbody>
</table>
O. *In vitro* anchoring performance of barbed sutures measured in the Full Factorial Design III (n = 6, Section 5.3.2)

<table>
<thead>
<tr>
<th>Category</th>
<th>Ultimate load to failure (N)</th>
<th>Load at 2-mm gap (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suture material</td>
<td>PDO</td>
<td>P4HB</td>
</tr>
<tr>
<td>Size 0</td>
<td>87.06 (20.4)</td>
<td>56.03 (11.59)</td>
</tr>
<tr>
<td>Size 2-0</td>
<td>89.19 (14.86)</td>
<td>56.58 (13.31)</td>
</tr>
<tr>
<td>Size 3</td>
<td>60.42 (20.57)</td>
<td>79.75 (27.33)</td>
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P. *In vivo* anchoring performance (N) of barbed sutures measured as the maximum pullout force in the rat model (n = 10, Section 6.3.1)

<table>
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<th>Days</th>
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<th>14</th>
<th>28</th>
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<tbody>
<tr>
<td>PDO</td>
<td>5.78 (0.71)</td>
<td>3.54 (0.38)</td>
<td>2.40 (0.57)</td>
<td>2.25 (0.79)</td>
<td>1.38 (0.77)</td>
</tr>
<tr>
<td>P4HB</td>
<td>3.28 (0.86)</td>
<td>1.73 (0.34)</td>
<td>1.50 (0.43)</td>
<td>1.73 (0.79)</td>
<td>2.40 (0.67)</td>
</tr>
</tbody>
</table>
Appendix B

Infiltration, embedding and polymerization of tissues in glycol methacrylate (GMA), and H&E staining protocols

Safety requirements: hooded working area, gloves at all times

Technovit® 7100 embedding kit from Electron Microscopy Sciences, Hatfield, PA

- Technovit® 7100 base liquid (2-hydroxyethyl methacrylate) store in fridge
- Hardener I (benzoyl peroxide) store in fridge
- Hardener II (dimethyl sulfoxide)

Glycol methacrylate (GMA) infiltration

Tissues are fixed in neutral buffered formalin.

1. Begin dehydration prior to infiltration. Under gentle vacuum for one hour each, run tissues through a series of changes in ethanol: 60%, 70%, 80%, 95% and a second 95%.

2. Prepare infiltration solution: add 1 g Hardener I to 100 ml Technovit® 7100 base liquid in 150 ml brown bottle. Vacuum and agitation are helpful. At 4 °C the infiltration solution remains stable for approximate 4 weeks.

3. Three changes of infiltration solution follow the ethanol series. Use enough solution to cover the tissue + 1/3 tissue volume. Begin with 30 – 60 minutes under gentle vacuum in the first change of infiltrate. Remove from vacuum and allow infiltrating at room temperature overnight. Tissue will likely float in the first infiltration solution.

4. Repeat each following day with another change of infiltration solution with the first hour under gentle vacuum.
5. In the afternoon of the third day (third change), tissues can be embedded and polymerized. Tissues should be fully sunk in the infiltration solution.

6. If tissues cannot be embedded right away, they can remain in the infiltration solution in the refrigerator indefinitely. If the infiltration solution begins browning, it is no longer good for embedding.

**Glycol methacrylate (GMA) embedding and polymerization**

Materials: clean embedding molds, metal blocks, 50 ml disposable beakers, forceps, plastic pipettes, glass pipettes and bulbs, toothpicks, box for storage during overnight polymerization, ice bath, and overhead light.

1. Prepare embedding solution in 50 ml beaker: 15 ml infiltration solution to 1 ml Hardener II. Make enough for the number of tissues being polymerized. Placed in ice bath for the duration of embedding process.

2. Place clean metal molds in the box.

3. Label stickers and place on the metal blocks.

4. Fill bottom of the mold with a thin layer of embedding solution. Add the tissue and orient for longitudinal and cross-sectional sections. Tissues should not touch the edge of the mold. Fill the mold with additional embedding solution to the first lip.

5. Wait until tackiness and monitor orientation. Test with the forceps like checking a cake. The toothpick should pull a string. At initial tackiness, add more embedding solution to bring to the second lip. Place the metal block over the tissue. Place a few drops in the stem of the block to form a seal.
6. Monitor tackiness every 5-10 minutes around the base of the blocks. At base tackiness, a good seal should have formed between the mold and the metal block. Begin filling the stems with embedding solution. Monitor continuously until the stems hold the embedding solution. Embedding solution can be removed from the base of the block and added to the stem until a good seal is holding. Discourage air bubbles by sticking the toothpick into the stem.

7. When the stem is full and holding, lid the box and leave overnight.

8. The following day, unmold the blocks and place stem down in the 65 °C oven for one hour.

9. Place directly into a desiccator box for storage.

**Hematoxylin & Eosin (H&E) staining**

<table>
<thead>
<tr>
<th>Step</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills Hematoxylin</td>
<td>17 minutes</td>
</tr>
<tr>
<td>Rinse in distilled water</td>
<td>20 dips</td>
</tr>
<tr>
<td>Scotts tap water</td>
<td>2 minutes</td>
</tr>
<tr>
<td>Distilled water</td>
<td>10 dips</td>
</tr>
<tr>
<td>Buffered Eosin-Phloxine</td>
<td>6 minutes</td>
</tr>
<tr>
<td>Distilled water</td>
<td>5 dips</td>
</tr>
<tr>
<td>100% ethanol</td>
<td>5 dips</td>
</tr>
<tr>
<td>100% ethanol</td>
<td>5 dips</td>
</tr>
<tr>
<td>50/50 ethanol xylene mixture</td>
<td>5 dips</td>
</tr>
<tr>
<td>Xylene</td>
<td>2 changes</td>
</tr>
</tbody>
</table>