DRUG LOADED POLYMERIC BLENDS FOR DEVELOPING VASCULAR STENTS

by

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ABSTRACT

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In-stent restenosis and neo-intimal hyperplasia are long-term limitations of metallic stents. Local delivery of medications to transluminal angioplasty sites to prevent restenosis is receiving worldwide attention. Development of localized application of anti-proliferative agents, and gene therapy via bio-degradable coatings on metallic substrates proved to be successful in reducing restenosis but was limited by the problems of limited drug load, etc. The presence of a foreign object for a long period of time may lead to the further narrowing of the treated lesion. The rationale for usage of bioresorbable drug eluting stents is the ability to store large quantities of drug and deliver measured quantities of the drug during the entire time the stent is present. This way one could hope for efficient repression of the proliferation of smooth muscle cells, effectively leading to control over restenosis. One might also enhance the endothelialization of the treated stent by absorbed promoters in the stent fiber matrix.

The aim of the study was to modify the mechanical characteristics, improve degradation performance and develop a curcumin loaded stent with favorable elution characteristics on the biodegradable PLLA based stent designed by Su [1].

We have achieved the desired control of the polymeric substrate by modifying and re-designing the bioresorbable fibers, employing different polymer blends of various compositions. The degradation rate of the optimal blend was 12% loss of weight over 4 weeks incubation in saline at 37 Deg C. The thermal analysis showed a melt temperature of 169 Deg C, thereby allowing loading of curcumin in the stent matrix during melt extrusion. We mechanically tested stents made from various polymer blends. The stent inflation pressure ranged from 3-5 atmospheres. Recoil following incubation in 37 Deg C saline varied from 2-10 % over a period of 1-4 weeks, depending on polymer blend. We found that most of the results in regards to stent performance testing were comparable to the mono-polymer stents designed and developed by Su [1] and Satasiya [2].

We conclude that a stent made of selected polymer blends could perform better in many aspects, including drug elution, inflammatory response and mechanical performance than previous drug-eluting bioresorbable stent models.

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INTRODUCTION

A biomaterial by definition is “a non-drug substance suitable for inclusion in systems which augment or replace the function of bodily tissues or organs”. From as early as a century ago artificial materials and devices have been developed to a point where they can replace various components of the human body. These materials are capable of being in contact with bodily fluids and tissues for prolonged periods of time, whilst eliciting little if any adverse reactions. Bioresorbable refers to a material that upon placement within the human body starts to dissolve (re-sorbed) and slowly replaced by advancing tissue.

1.1 History of percutaneous transluminal coronary angioplasty (PTCA):

Cardiovascular diseases are considered to be the principal causes of death within United States, Europe, and much of Asia [6]. Atherosclerosis, a disease of the large arteries, is the primary cause of heart disease and stroke [7] High plasma concentrations of cholesterol, in particular those of low-density lipoprotein (LDL) cholesterol, are one of the principal risk factors for atherosclerosis [5]. Accumulation of lipids and other prothrombotic elements into the liquid cores of “vulnerable plaques” found in vessel walls in advanced stages of atherosclerosis is considered to be one of the principal ominous features in the evolution of the disease (Fig. 1.1). It seems that these plaques are often result in chronic inflammation condition is which is converted to an acute clinical event.

The induction of plaque rupture, which in turn leads to thrombosis, ischemia, and potentially, myocardial infarction [9]

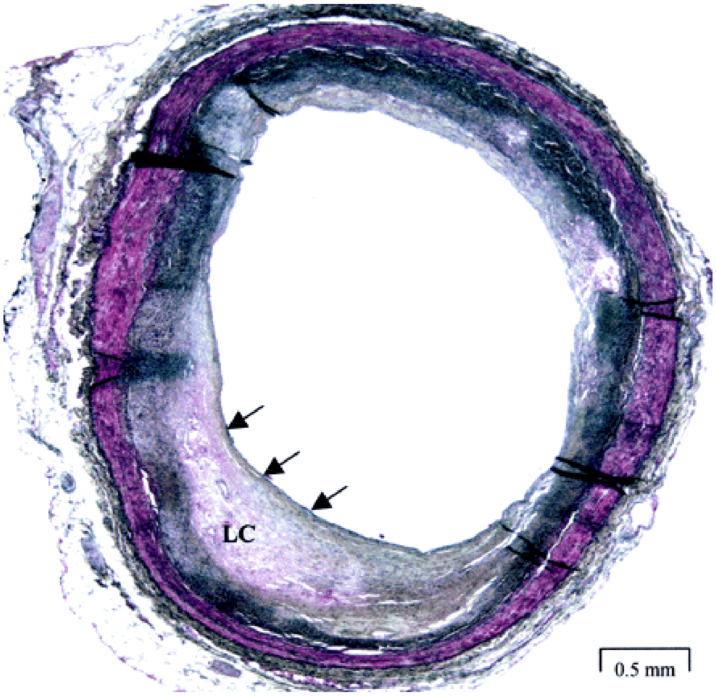


Figure 1.1. Histology slide of vulnerable coronary plaque. Note the thin fibrous cap (arrows) and lipid core (LC), important features of vulnerable plaque. Of note, the preserved luminal area renders coronary angiography or other forms of luminography obsolete in vulnerable-plaque detection.

In 1964 Charles Dotter and Melvin Judkins described a new technique for relieving stenosis of the iliofemoral arteries with rigid dilators [4]. In Zurich, Andreas Gruentzig substituted a balloon-tipped catheter for the rigid dilator and performed the

1st peripheral balloon angioplasty (PTCA) in a human being in 1974 [4]. PTCA in these early series consisted mainly 3 primarily steps: 1) Insertion of a catheter through an artery in the groin or arm. This permits relatively ease of advancement of a guidewire to the coronary vessels; 2) The deployment of a smaller catheter with a balloon tip, over the guidewire into the narrowed coronary artery; 3) Inflation of the balloon once the catheter was properly positioned across the stenotic region. This expansion permitted breakup of plaque material, and expansion of the unaffected portion of the vessel wall, leading to an increased cross section that allowed better blood flow.

As noted, the increase in the lumen diameter post-PTCA was attributed to compression of the atherosclerotic plaque. However, other possibilities include redistribution of the atheroma, embolization of atherosclerotic debris, stretching or aneurysm formation, and localized fracture or dissection of the plaque [13, 14, 2]. This barotrauma-induced injury [2] to the lumen of the vessel wall results in triggering of the complex biological "healing" process, thought to be accomplished within the first six months following PTCA. However in a large number of cases (15-30%) it also resulted in renarrowing of the artery- restenosis [10]. Due to this reason there was a higher chance of repeated PTCA procedures then initially expected. Nevertheless, during the first decade of angioplasty, improvements in equipment and techniques resulted in an enhanced clinical success (post PTCA stenosis <50% with no in-hospital death, MI, or

(CABG) of 83% vs. 55%, leading to improved 5 year outcomes for patients treated during the latter period [12,2].

1.2 The Evolution of Stents

The term "stent" initially defined a dental support device [1, 2, 16] developed by Charles Stent. It later became associated with a device that held a skin graft in position, then a support for tubular structures that were being interposed in blood vessels (anastomosed) and, more recently, an endovascular scaffolding to relieve and prevent vascular obstructions [16].

By 1997, angioplasty had become one of the most common medical interventions in the world.[4] and had become a critical technique in the treatment of coronary heart disease [1]. As noted, PTCA continued to evolve but was limited by the problems of vessel closure, both acute thrombotic episodes during the original intervention, and subsequent longer term restenosis, requiring follow-up. Thus PTCA continued to evolve and subsequently led to applications such as coronary atherectomy (1986) and coronary stenting (1987) [6]. Dotter et al during the early 1980s developed expandable nitinol based coils that could be expanded by circulating heated saline solution through the catheter, allowing the coil to enlarge to its predetermined form with a luminal diameter approximately equal to that of the adjacent blood vessel [16]. This stent would radially buttress the dimensions of the vessel wall to treat luminal narrowing [2]. The first stent implantation, the "Wallstent" by Sigwart et al (1986) in Europe [1] lead to the rapid approval, by the FDA, of intracoronary stent Phase I trials of various stent designs to assess their efficacy in conjunction with PTCA. Researcher's Roubin and Schatz [2] performed the first stent deployment in the United

States. The approval of the Gianturco-Roubin stent in 1993 and Palmaz-Schatz stent in1994 lead to the rapid adoption of stents in clinical practice in the USA and subsequent tremendous improvements in stent design and deployment technology [1,2,].

Today, in most centers in the United States and Europe, stents are being used in over 70% of percutaneous coronary interventions. Stent-assisted PTCA has significantly reduced the need for acute revascularization surgery [21], leading to major adjustments in coronary patient treatment patterns. In the last decade metal stent performance has been improved considerably, by design changes improving flexibility and deliverability, visualization, stability, lack of thrombogenicity and, to a certain extent lowering cost. Today there are more than 40 types of stents that are commercially available [2, 21].

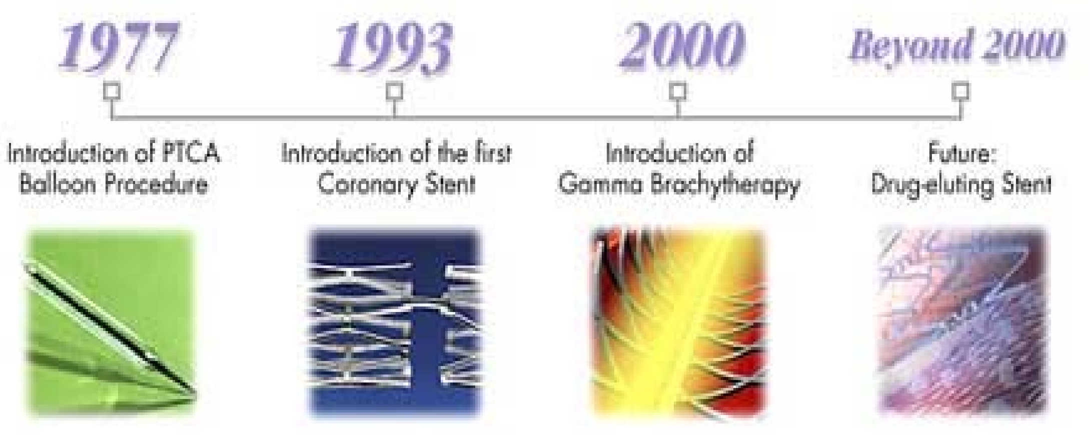


Figure 1.2 History of Stent Development

Most of the patented and commercially available stent designs are based on a fixed diameter deflated stent mounted on a balloon catheter that can deployed to a specific diameter to expand the vessel cross section and thus provide a first line treatment of coronary diseases. The deployment protocol followed by interventional cardiothoracic surgeons is the use of inflatable balloon mounted on a catheter that is passed to the lesion or blockage where it is inflated simultaneously with the stent to hold the artery open. Once the stent is positioned then the balloon is deflated thus minimizing the occlusion and increasing the flow through the artery.

1.3 Types of Stent Designs:

In general, stent designs can be classified into balloon expandable, self-expandable, coated, and drug-eluting categories. Commonly, stents are manufactured with materials that are biologically inert, in order to lessen the initiation of biological reaction.

Table 1.1 commonly used stents in the US market for treating coronary and peripheral vascular diseases.

Category	Type	Structure	Distributor/ Manufacturer
Tubular	Palmaz-Schatz (J&J) Stent	Slotted tube	Johnson & Johnson/Cordis Warren, NJ
	CrossFlex LC Coronary Stent	S-waved slotted tube	Johnson & Johnson/Cordis Warren, NJ
	Palmaz-Schatz Crown Stent	Slotted tube with longitudinal sinusoidal configuration	Johnson & Johnson/Cordis Warren, NJ
	AVE Micro Stent II	Tubular strut composed of numbers of continuously connected sinusoidal elements	Medtronic/AVE Santa Rosa, CA
Coil	Gianturco-Roubin FLEX Stent	Coiled wire	Cook, Inc. Bloomington, IN
	Wiktor	Helical coil composed of waved single wire	Medtronic Minneapolis, MN
	NIR	Flexible coil	Boston Scientific/Scimed Natick, MA

1.3.1 Metal Stents:

Metal stents evolved from relatively stiff, difficult to deploy structures, designed to prevent wall dissection and collapse, to more flexible, open architectures which a trained interventional physician or surgeon can navigate into tortuous channels and also to overlay vessel branches whilst maintaining their patency [17]. The Wallstent (Schneider), Palmaz-Schatz (Johnson & Johnson), Wiktor (Medtronic) and Gianturco-Roubin (Cook) were some of the second generation stents that evolved from early designs [2]. These material used for manufacturing of stents were stainless steel, Nitinol® or platinum alloy. The clinical trails after FDA approval of these stents improved on the performance of PTCA-only procedures [2]. However they were also limited by in-stent restenosis, stent induced thrombosis, and inflammation.

1.3.2 Polymer- and Metal-Coated Metallic Stents

In order to modify the early thrombosis as well as the late neo-intimal proliferation associated with PTCA and stenting, polymeric coatings of metal stents, both permanent and degradable were studied extensively, for two reasons: 1) to improve surface properties; 2) to permit loading of drugs into the polymer matrix [21]. Many different materials and techniques were used to cover the stent surface, and an array of application techniques were employed, such as dipping, (plasma-) spraying, plating, sputtering, and surface induced mineralization [21]. Four common approaches were used in early investigations to reduce the in-stent restenosis [1]. Gold, being biologically inert, was used as a stent coating material in order to gain control over restenosis, with mixed results [18]. Heparin coated stents [19] and phosophorylcholine-coated stents [20], both based on polymeric coatings, were also not successful in reducing the restenosis and neointimal hyperplasia. More recent developments in stent coatings have turned towards inorganic materials such as silicon-carbide (SiC) or iridium oxide: these are reported to have minimal bio-compatibility issues [22].

1.3.3 Drug Loaded Stent Coatings:

The migration of vascular smooth muscle cells to- and growth at the vessel wall adjacent to the stent surface after vascular injury results in neointimal proliferation and is a key mechanism of in-stent restenosis. Inert coatings of stents and other mechanical approaches have proved to be too simplistic to prevent in-stent restenosis. Interfering with cell division by various molecular approaches appears to be a much more effective strategy to alter the healing process after stenting [24]. Systemic pharmacology was ineffective due to the potential toxicity and the failure to achieve adequate drug concentrations at the injury site [24]. This led to the concept of local drug delivery via the stent.

As these disappointing results became known, the concept of using stents as vehicles for prolonged intramural drug delivery was studied extensively. Stents present an attractive platform for local drug delivery due to their permanent scaffolding property, thus preventing vessel recoil and negative remodeling. In addition, the coatings can serve as reservoirs for drug release over various time intervals. A drug-eluting stent can be defined as a device capable of releasing therapeutic agents in into the bloodstream that can affect tissues around the stent. The methods of the delivery of the bioactive agent can be through the stent surface, embedded and released from within polymer matrix linked on the stent surface. The research into different anti-proliferative agents like, paclitaxel, sirolimus, curcumin [1, 2] is under investigation for their safety and efficacy in the treatment of coronary lesions [47]. Some of the systemic drug choices that are under investigation include lipid-lowering agents, anti-thrombotic agents, angiotensin converting enzyme (ACE) inhibitors, calcium antagonists, β-blockers, rapamycin, dactinomycin, QUADS-QP2 taxane and batimatast. They proved effective with some adverse events, such as late thrombosis, delayed restenosis and aneurysm formation. [48, 49]. Usage of polymer coatings did not prove as effective since by their nature coatings typically induce inflammatory responses and fibrinoid deposits along with delayed intimal hyperplasia.

Morice et al published the first clinical experience with drug-coated stents employing the anti-proliferative drug rapamycin (Sirolimus), previously used to control organ transplant rejection [23]. In a trial of 238 patients, stenosis at 6 months (>50% of the vessel lumen) was 0% with sirolimus-eluting coated stents as opposed to 27% for normal stents ($p<0{\cdot}001$). The SIRIUS trial, a subsequent study that randomized 1100 patients to treatment with a rapamycin-coated stent or an uncoated (standard) stent, investigated the long-term safety of stent-based drug elution in complex coronary lesions. Preliminary results showed a significant reduction of in-stent restenosis (3.2% drug-eluting stent *vs* 35.4% standard stent) and in segmental restenosis (8.9% *vs* 36.3%), [23]. Although these stents have performed much better in terms of restenosis, they should be used with caution, as there are several reports of thrombosis-associated deaths of drug eluting stent recipients, which raised concerns by the FDA and European regulatory agencies [2]

1.3.4 Bioresorbable Stents

Current clinical data suggests the success rate of the current metallic stents is over 90% in treatment of occluded vessels [31] but such vessels remain open to sub-acute thrombosis although improved deployment techniques and anticoagulation regimens have reduced both the rate of acute thrombosis and aggressive anticoagulation. Acute closure and restenosis commonly occur 3 -6 months [27] following coronary intervention. As they being permanent fixtures they proved to be obstacles for repeat procedures such as repeat angioplasty and bypass surgery [27]. Theoretical concerns were also raised with regard to flexibility mismatch between the stent and the compliant vessel wall, and with the potential for chronic vessel wall trauma which may predispose the vessel to medial atrophy, aneurysm formation, and/or late reactive hyperplasia.[32]

Metal stenting freezes recoil to a large extent, but it also freezes remodeling. Chronic arterial responses to metal stents have been studied with intravascular ultrasound, and it seems that metal stents prevent the lumen expansion associated with late favorable remodeling, despite allowing some enlargement of the vessel surrounding the stent. [29]. The possibility of replacing a permanent metallic implant may permit remodeling with lumen enlargement to compensate for the development of new lesions was the basis on which the biodegradable stent was designed. [27].

Stack et al at Duke University developed the first biodegradable stent and implanted it in animals [30]. A degradable polymer of poly-L-lactide was used for this prototype stent, which could withstand up to 1000 mm Hg of crush pressure and keep its radial strength for 1 month and could degrade relatively quickly. This device showed the presence of minimal thrombosis, moderate neointimal growth, and a limited inflammatory response in the early animal implants [27, 30].

Biodegradable polymer stents in theory could provide temporary mechanical support for the vessel wall from the time of transluminal revascularization until remodeling processes have stabilized the instrumented vessel. Subsequent bioresorption of the polymer might eliminate potentially deleterious long-term effects. In addition, the porous structure of the polymer stent could provide a reservoir for local delivery of therapeutic molecules or particles to the vessel wall [32] were some of the potential advantages that resorbable stents provided.

Potential applications of bioresorbable stents have been curtailed by the difficulties associated with the development as compared to the simplicity and low cost associated with manufacturing stainless steel stents. [27].

1.4 Advantages and Disadvantages of Stenting

1.4.1 Advantages

Although being introduced only in the ‘90s, over 400,000 percutaneous transluminal coronary (PTCA) procedures are performed annually in the United States, with primary success rates approximating 90% [32]. The expanded utilization of stents raised concerns about patient screening for the decision for PTCA, stenting, or emergency coronary artery bypass grafting [2]. Based on the recommendations of the

American College of Cardiology/American Heart Association, the decision for indicated lesions for stenting was classified according to lesion type, concluding that intra-coronary stenting could be implemented in patients with the following lesions: 1)Favorable lesions; 2) Restenotic lesions; 3) Acute or threatened closure; 4) Lesions in saphenous vein grafts (SVG); 5) Aorto-ostial Lesions; 6) Left main coronary artery lesion (LMCA); 7) Chronic total occlusions; 8) Acute myocardial infarction.

Following successful balloon angioplasty, stents can prevent late vessel remodeling (chronic vessel recoil) by mechanically reinforcing the vessel wall and resetting the vessel size resulting in a low incidence of restenosis [2]. Studies performed related to stenting and angioplasty and revealed patients who underwent stenting had a larger immediate increase in the diameter of the lumen after the procedure than those who underwent standard balloon angioplasty [33].

Table 1.2 Lesion characteristics: Type B1 lesions have one 'B' characteristic but type B2 has two or more 'B' characteristics. Source: ACC/AHA Task Force Report. Guidelines for Percutaneous Transluminal Coronary Angioplasty. J Am Coll Cardiol 1988; 12: 529-545 [2]

	A	B*	C
Length	Less than 10 mm	10-20 mm	Greater than 20 mm
Shape	Concentric	Eccentric	
Angulation	Less than 45°	Greater than 45°, less than 90°	Greater than 90°
Tortuosity of proximal segment	None or minimal	Moderate	Excessive
Calcification	None or minimal	Moderate to heavy	Moderate to heavy
Occlusion	Non-occlusive	Total occlusion less than 3 months old	Total occlusion older than 3 months
Ostial	No	Yes	Yes
Bifurcation	No	Yes, but protected with double guide wires	Yes, and unable to protect major side branches
Thrombus	None	Some	Some
Other factors	No	No	Old saphenous vein graft

A timed study was conducted, showing that the occurrence of restenosis at six months was lower in stented vessels as compared to treatment with balloon angioplasty, while maintaining the a larger lumen. The success rates of the angioplasty groups were enhanced by crossover to stenting in an average of 5 % of patients. Stenosis and the need for repeat intervention were reduced with stenting after 6 months of follow-up but rates of death, MI, or emergent CABG were comparable with both strategies. These findings were also confirmed at one year follow-up done by the same researchers [33, 34, 2].

1.4.2 Disadvantages

It is now well accepted that stent placement effectively reduces the risk of acute closure and late restenosis after the intervention procedure [1]. However stenting is limited in small vessel (<3 mm) lesions, in which there are concerns with higher rates of stent thrombosis and restenosis 2) long lesions and diffuse disease 3) bifurcation and ostial lesions, which are technically challenging in terms of stent design and deployment 4) unprotected left main stenting and 5) multi-vessel stenting vs. CABG [35]. Clinical data published in the recent past reveals many cases where in the lesions are stable and the lumen size hasn't changed by a significantly. Vascular injury during the deployment phase defines the extent of impact on thrombosis and in-stent restenosis and hence has been in main focus of stent work in recent years. They are also limited by site navigation difficulties in extremely tortuous vessels; they are also prone to thrombus formation.

It has been demonstrated in clinical trials that stents prevent elastic recoil following balloon angioplasty, but may be compromised by late restenosis, subsequent to neo-intimal hyperplasia [27,29]. A "brachytherapy" application was developed in an effort to limit the hyperplastic response [10]. It was noted that interventional procedures employing stents loaded with low dose beta emitters seemed to limit neo-intimal hyperplasia, but at an unacceptable cost: radiation-induced damage to the vessels.

Metal stents are also limited in terms of drug loading to combat thrombogenesis and neo-intimal hyperplasia. In order to develop better drug loading capacity, stent coatings with degradable polymers containing drugs were developed the late 20th century and early 21st centuries. Clinical trails showed the quick release of drugs was effective in reducing the hyperplastic response, but the effect was criticized on theoretical grounds, due to the limited drug loading capacity of the coatings. Recently other stent designs have been developed to increase the capacity and range of delivered drugs, e.g., the Conor stent.

Stents coated with biocompatible materials containing anticoagulants, and corticosteroids have been examined in both animal and human studies. These studies suggest that stents coated with these agents do not have a significant inhibitory effect on neointimal hyperplasia [36]. Thus other, "antiproliferative" drugs were examined, as noted previously. It is important to note that the long-term effects of bare metal stents and drug-eluting stents with biodegradable coatings in humans are still to be determined.

1.5 Rationale for Bioresorbable Stents – Usage as a Drug Delivery Vehicle

Published data from the clinical trials has proved that metallic stents appear well tolerated but recent histological evidence relates chronic inflammation and injury with the permanent residency of the stent [11,31,33,34]. Coronary dissections are effectively contained by stent insertion and undergo a healing process, with the majority of cardiac events occurring in the first 6 months [37] amongst which in-stent restenosis is a major occurrence. Hence these would put into picture the effect of the permanent status of the stent inside the vessel. Removal of the implanted stent surgically is extremely hazardous and impractical as it encounters serious trauma to the vascular wall. A support structure which exhibits mechanical strength to withhold the pressures exerted by the vessel wall could be designed with natural degrading capabilities would be far more favorable then surgical removal. The rationale for bioresorbable stents is the support of a body conduit only during its healing process. The natural degradation process will lead to the loss of stent mass and radial decrease in strength with time. However the hypothesis was that the mechanical load imposed on the stent will be, with time, transferred to the surrounding tissue. The Tianjin/Beijing stent and the Kyoto University PGA stent are some biodegradable stents that have been studied in animals. They show mild neointimal proliferation and mild thromobogenesis [24]. The Igaki/Tamai bioresorbable PLLA zigzag coil stent, that required heating and pressurization during the deployment phase was the first bio-resorbable stent placed in the human coronary artery. Clinical data at 6 months were quite encouraging [28]. Longer-term results have been reported regularly at the TCT annual meeting, the most recent indicating that the restenosis rate is no different than that for metal stents at 3 years.

1.6 Drug Eluting Bio-Resorbable Stents:

Coated stents faced limitations in the Drug loading capacity and not much control over thrombosis and inflammation and induce endothelial dysfunction was observed as a result of metal stents [32]. Controlled release of an active agent from a stent can be used to enhance healing of the surrounding tissues and to increase the implant’s biocompatibility. Bioresorbable stents also enable longer term delivery of drugs to the conduit wall from an internal reservoir with the additional assistance of curing certain diseases. Yamawaki et al were the first to incorporate an anti-proliferative agent into the high-molecular weight PLLA Igaki/Tamai stent [40]. Su, and later Nguyen, in our group, were successful in loading curcumin into poly-(L lactic acid) polymer fibers by melt extrusion. Zilberman et al showed that drug incorporated micro-spheres can be loaded successfully onto degradable polymer fibers [24].

Our group has also been successful in loading genes and delivering them to the arterial walls in rabbit and porcine models [32]. The possibility of transferring genes that code key proteins in the central regulatory pathways of cell proliferation inside the cells of the arterial wall using biodegradable stents as vehicles is exciting. Regardless of which agent (gene or drug) will finally conquer restenosis, a bioresorbable stent remains the optimal vehicle for such delivery.

Selection of the biocompatible polymer material is critical for the fabrication of a bioresorbable polymeric stent. Natural polymers and synthetic polymers are commercially available. . Natural polymers have potential as signaling molecules that could be tailored for specific cell responses, but they are not mechanically strong enough to maintain vascular structure. Synthetic polymer polymers can have much higher mechanical strength and their mechanical and other properties can be manipulated with ease by adjusting chain length, side chain and amount of cross-linking. Several types of commonly used polymers have been briefly introduced [1,24] and are outlined below:

1) Poly (α-Hydroxy Esters): These are the first polymers to be approved by FDA for implantation in human body in the form of degradable sutures. Poly-(L lactic acid) (PLLA) and poly (glycolic acid) (PGA) and their copolymers poly (lactic-co-glycolic acid) (PLGA) are the most frequently employed polymers of this family. Various copolymer ratios, degree of crystallinity, and chain length alter the mechanical properties and degradation behavior of this polymer. PGA is hydrophilic resulting in faster hydrolytic degradation where as PLLA, being

hydrophobic, degrades slowly. PLLA shows better solubility in solvents but the hydrophobicity makes such polymers more brittle [1,2,41

2)]. It is noteworthy that there is no linear relationship between the ratio of glycolic acid to lactic acid and the physico-mechanical properties of the corresponding copolymers. Since lactic acid is a chiral molecule, it exists in two stereo-isomeric forms which give rise to four morphologically distinct polymers: Poly-L-lactic acid (PLLA), Poly-D-lactic acid (PDLA), the racemic form (PDLLA) and meso-PLA. PDLA is the more amorphous form; the absence of crystallites makes the polymer weaker, mechanically. Poly (ε-caprolactone) (PCL) is also a member of this polyester family. PCL degrades mainly in bulk hydrolysis. Some enzymatic cleavage, possibly by nonspecific esterase, is involved in the degradation [42]. It degrades at a slower rate than PGA but has similar elastic properties.

(2) Poly (Ortho Esters): This amorphous hydrophobic polymer group is sensitive to hydrolysis only at lower pH conditions. Poly (ortho esters) exhibits a large range of mechanical properties and undergoes degradation by surface erosion phenomenon.

(3) Poly (anhydrides): This group also undergoes surface erosion. These polymers can be made aliphatic or aromatic, resulting in a large range of degradation rates from days to years. These polymers are brittle and have poor mechanical properties.

(4) Poly (amino acids): Poly (amino acids) is made of linkages to pendant functional groups either to the carboxyl group or to the amine or to other groups. Varieties of amino acids have been assembled both with the usual amide linkages using 20 natural

amino acids as well as others. Their degradation is usually hydrolysis of their hydrophobic side groups and enzyme-dependent [1].

(5) Polyphosphazenes: This is a group of inorganic polymers whose backbone consists of nitrogen-phosphorus bonds. These polymers have unusual material properties and have found industrial applications.

Of the mentioned biodegradable polymers, poly (α-hydroxy esters) are the mostly frequently used for applications in weight bearing implants designed to provide mechanical support, e.g., bone grafts and fracture fixation. Blends of PLLA/ PGA or PLLA/PCL and many more can be designed, but do not sustain the necessary mechanical strength needed for such implants. PLLA is the preferred because of its superior mechanical strength. Accordingly, PLLA is widely used in devices for orthopedic surgery and in other surgery fields where bioresorbable sutures are needed, and also is used for drug delivering implants. Agarwal et al was the first to suggest the usage of PLLA in for vascular grafts [43].

Some of the characteristics of these aliphatic polyester polymers are listed below [2]. Poly (L-lactide) (PLLA) is known to be the most desirable biocompatible and biodegradable semi-crystalline polymer obtainable from starch in high yield [46].

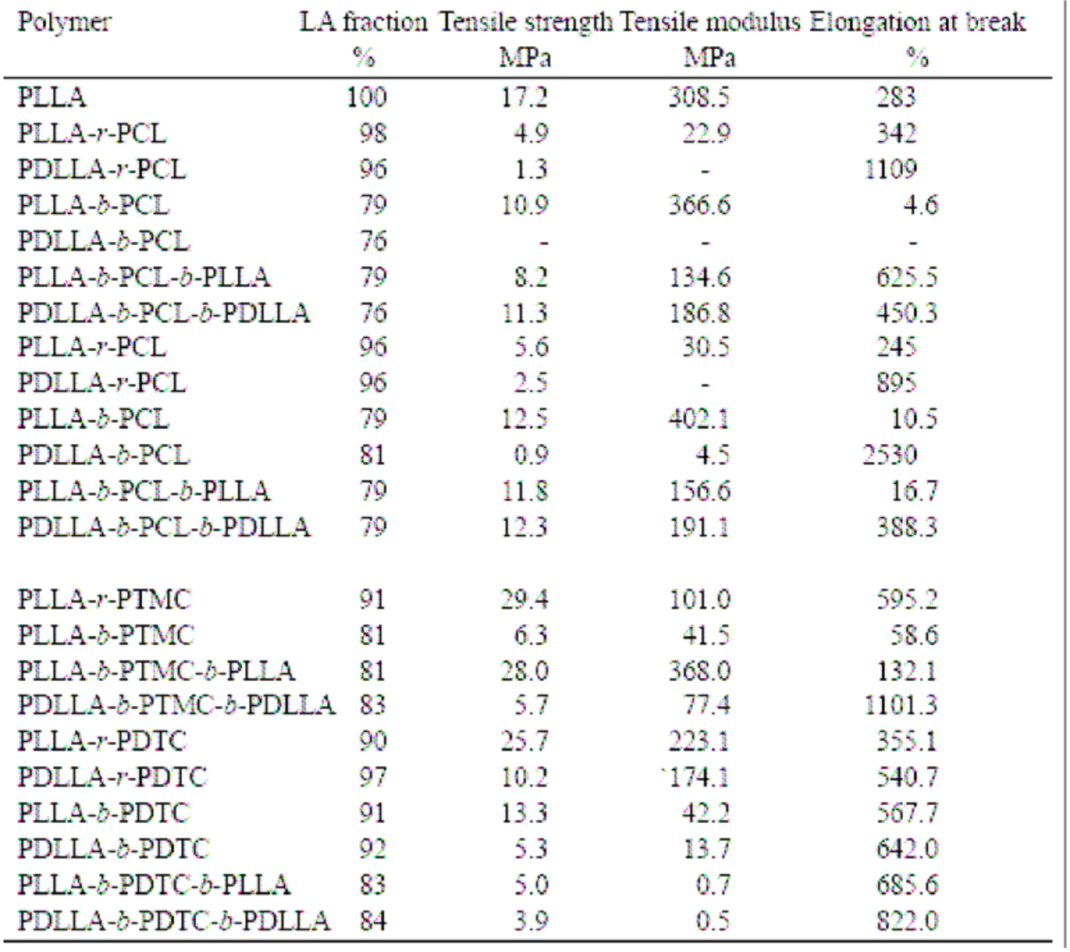
Table 1.3 Commercially available aliphatic polyester polymers

Polymer	Melting Point (ºC)	Glass Transition Temperature (ºC)	Modulus (GPa)	Degradation (months)
PGA	225-230	35-40	7.0	6-12
PLLA	173-178	60-65	2.7	>24
PDLA	Amorphous	55-60	1.9	12-16
PCL	58-63	-65 to -60	0.4	>24

These characteristics warranted the use of PLLA for biomedical applications, particularly those that demand good mechanical properties. However, in recent years, PLLA homopolymer has been reported to exhibit relatively poor biodegradability. Furthermore, PLLA homopolymer is found to exhibit too hard and too brittle characters as widely usable biodegradable materials. Therefore, physical properties must be improved by copolymerization of lactide with other monomers in order to generate more flexible materials. Random and block copolymerization of lactide with other monomers such as ∑-caprolactone (CL) and cyclic carbonates (CC) were performed using rare earth metal complexes and tin compounds.[45] Scientists from Japanese Hiroshima University, Belgian University of Liege devised and designed using different techniques as detailed in [45] resulting in PLLA-PCL blends that exhibited a higher degradability. Studies of the biocompatibility of these polymers were performed at Tufts University, and favorable results were obtained.

Table 1.4 lists some of the commonly used blended aliphatic polyester polymers for biomedical applications. The table describes the mechanical characteristics of these polymers as compared with PLLA. PLLA and PDLA have high tensile strength, permitting robust mechanical design, but typically have long degradation times, often an undesirable feature, as in vascular prostheses. PGA and PCL have less strength, but faster degradation rates.

Table 1.4: Development of highly degradable Lactide Polymers



Polymer	LA fraction %	Tensile strength MPa	Tensile modulus MPa	Elongation at break %
PLLA	100	17.2	308.5	283
PLLA-*r*-PCL	98	4.9	22.9	342
PDLLA-*r*-PCL	96	1.3	-	1109
PLLA-*b*-PCL	79	10.9	366.6	4.6
PDLLA-*b*-PCL	76	-	-	-
PLLA-*b*-PCL-*b*-PLLA	79	8.2	134.6	625.5
PDLLA-*b*-PCL-*b*-PDLLA	76	11.3	186.8	450.3
PLLA-*r*-PCL	96	5.6	30.5	245
PDLLA-*r*-PCL	96	2.5	-	895
PLLA-*b*-PCL	79	12.5	402.1	10.5
PDLLA-*b*-PCL	81	0.9	4.5	2530
PLLA-*b*-PCL-*b*-PLLA	79	11.8	156.6	16.7
PDLLA-*b*-PCL-*b*-PDLLA	79	12.3	191.1	388.3
PLLA-*r*-PTMC	91	29.4	101.0	595.2
PLLA-*b*-PTMC	81	6.3	41.5	58.6
PLLA-*b*-PTMC-*b*-PLLA	81	28.0	368.0	132.1
PDLLA-*b*-PTMC-*b*-PDLLA	83	5.7	77.4	1101.3
PLLA-*r*-PDTC	90	25.7	223.1	355.1
PDLLA-*r*-PDTC	97	10.2	174.1	540.7
PLLA-*b*-PDTC	91	13.3	42.2	567.7
PDLLA-*b*-PDTC	92	5.3	13.7	642.0
PLLA-*b*-PDTC-*b*-PLLA	83	5.0	0.7	685.6
PDLLA-*b*-PDTC-*b*-PDLLA	84	3.9	0.5	822.0

It is important to note that PLLA, with a low molecular mass (around 80 kDa) is associated with an intense inflammatory reaction, whereas a minimal inflammatory reaction occurs with implants that are coated with high-molecular-mass (around 321 kDa) PLLA [44]. Different manufacturers use different methods for developing these polymers. This at times contains usage of additives, surfactants and other products to enhance the polymer properties such as miscibility, viscosity etc. Predominantly we have used Resomer L210 (Bohringer Ingelheim-Germany) as literature suggests it has shown to have a minimal inflammatory reaction [17]. Care has been taken to use polymers from the same company in order to minimize the deviations from the standard protocols followed to polymer manufacture.

1.6.1 Degradation Mechanism Of Polymers

In order to be sure that a bio-absorbable stent will not lose its radial strength before healing has occurred, it is important to determine how the degradation process affects the stent mechanical properties. Several authors have found no difference between *in vitro* and *in vivo* degradation but several authors have reported faster degradation *in vivo* than *in vitro*[62]. These findings have been explained by cellular and enzymatic activities within the body, and the dynamic load affecting implanted devices.

Langer et al describe several mechanisms that can affect the degradation rate of biodegradable polymers [55]. As shown in Figure 1.3, mechanism I is chemical reactions leading to cleavage of cross-links between water-soluble polymer chains. Mechanism II is cleavage of polymer side chains resulting in the formation of polar or charged groups. Mechanism III is the cleavage of the backbone within the chain. Combinations of these mechanisms are possible: for instance, a cross-linked polymer may first be partially solubilized by the cleavage of cross links (mechanism I), followed by the cleavage of the backbone itself (mechanism III) [2]. PLLA can degrade mainly by nonspecific scissions of the ester bonds in its backbone via hydrolysis. The final product (lactic acid) can enter the tricarboxylic acid cycle and be disposed of as carbon dioxide and water [59]. The cleavage of an ester bond yields a carboxyl end group and a hydroxyl one. The thus formed carboxyl end groups are capable of catalyzing hydrolysis of other ester bonds, a phenomenon called auto- catalysis [60].

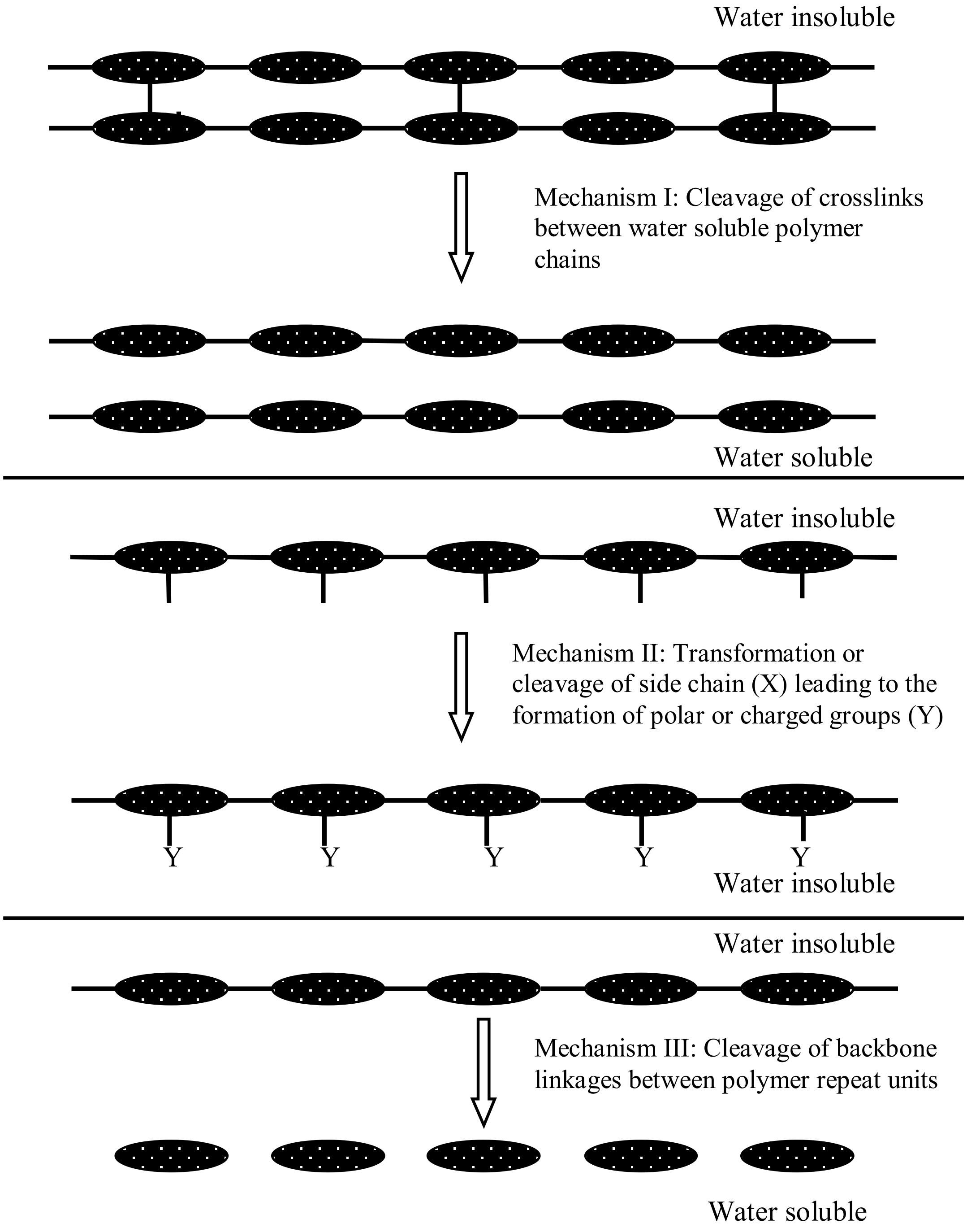


Figure 1.3 Cleavage mechanisms responsible for the degradation of polymers

1.7 Objective 1 Characteristics of Stents Designed from Polymeric Blends

The design and development of a naturally degrading vascular graft has been detailed in [1]. The stent design was improved as detailed in [2] for better grip qualities on the balloon catheter, mechanical properties, degradation profiles. Impregnation of a drug with minimal loss of the pharmacologic characteristics in the stent fibers had been done previously [1] in Low Molecular weight PLLA. Drug loading in high molecular weight PLLA did not produce successful results. Developing a polymeric blend that would modify the crystalline structure of high molecular weight PLLA, in turn assisting in the loading of curcumin in high-molecular weight PLLA, was attempted in the project. The feasibility of developing stents and testing various aspects such as a collapse pressures, mechanical strength and gluing methodology was performed. Thermal characterization was done in-order to determine melting points and glass transition temperatures of the blends.

1.8 Objective 2 Curcumin Loading in Fibers and Degradation Analysis

Metal and polymeric stents themselves trigger restenosis, a current clinical challenge. The potential usefulness of immunosuppressive agents in the treatment of restenosis arises from parallels between tumor cell growth and the benign tissue proliferation that characterizes intimal hyperplasia [47]. Localized delivery of therapeutic agents to the traumatic vessel showed promising results during its early trials. Avoiding systemic toxicity, stent-based local drug-release at the site of vascular injury via a polymeric-coated stent is an attractive therapeutic method to achieve an effective local concentration of drug for a designed period [47].

A drug-eluting stent can be defined as a device capable of releasing therapeutic agents in into the bloodstream that can affect tissues around the stent. The methods of the delivery of the bioactive agent can be through the stent surface, embedded and released from within polymer matrix linked on the stent surface. The powdered dry rhizome of the plant *Curcuma longa* Linn, commonly called turmeric has been used for centuries as a traditional medicine to treat inflammatory and other diseases [51]. Diferuloylmethane is the major yellow pigment in turmeric, curry and mustard. Commercial curcumin isolated from the powdered dry rhizome of *Curcuma longa* Linn contains approximately 77% curcumin, 17% demethoxycurcumin, and 3% bisdemethoxycurcumin [52, 53]. Isabel Brouet et al studied the anti-inflammatory and anti-tumor effect of curcumin on inhibition of nitric oxide on activated macrophages. L-arginine derived nitric oxide and its derivatives such as nitrogen dioxide and peroxynitrite. Her group concluded that small concentrations of curcumin were able to inhibit the activity of macrophages due to production of NO [54] as compared to other anti-inflammatory inhibitors such as aspirin, sodium salisylate, piroxicam, ibuprofane etc. Several other independent studies including work done by Su et al and Tang et al relates to the reduction the activation of macrophage activity by curcumin.

The rate at which a polymer degrades expresses control over the rate at which the drug is released into the surrounding medium. Factors such as chemical stability of the monomer, hydrophobicity, and molecular weight determine the overall rate of degradation process The adaptation to study the effects of different polymer blends and their degradation rates in an attempt to enhance release of curcumin in a shorter time period was emphasized in this objective.

1.9 Objective 3 Stent Integrity Analysis

The stent designed by Su and later modified for enhanced features by Satasiya [2] is the basis for this study. In that work, inherent built-in stresses generated during the manual fabrication process led to a triangular configuration rather than a circular configuration after deployment. An attempt was made in this study to release the built up stresses and shift the stent towards a circular configuration. The stent struts were "welded" to the axial fibers thus maintaining the stent integrity and increasing the ability of the stent to withstand mechanical pressure exposed to it during deployment phase. This particular gluing method led to formation of string like remnants near each and every strut joint. Experimental procedures were implemented to exhibit control over such thread like formation and minimize it during the stent fabrication.

CHAPTER 2

MATERIALS AND METHODS

2.1 Extrusion of PLLA Fibers:

The degradable stent developed by Su et al. was based on the creation of a coil-within-coil geometry, by weaving a continuous polymeric fiber around a series of co-parallel mandrels [2]. This approach involved creating loops of very small radius of curvature and snugging of the coil, both of which placed demands on fiber strength. These considerations, plus the small initial furled diameter geometry of the completed stent, and the forces on the fiber induced by expansion of the angioplasty balloon, dictate that mechanically strong and flexible polymer fiber is needed such that the fibers can be wound on the mandrel with ease.

2.1.1 Materials

Su et al. chose poly(l-lactic acid) as the polymer for stent fabrication, as it provided both the needed mechanical strength and the biocompatibility [1a]. It has been shown before that low molecular weight PLLA initiates a extremely intense inflammatory response [44], where as a minimal inflammatory response is reported using high molecular weight PLLA. Resomer® L210 (Bohringer-Ingelheim, Germany) was chosen for this purpose with a nominal molecular weight of 579 kD. The polymer was used as-is after receipt from the manufacturer. It was melt-processed in a batch extruder

(Alex James, Greer SC) located in the Surgery Department BME laboratory at UT Southwestern.

2.1.2 Fiber Extrusion Methodology:

The extruder ram employed to charge the cylinder and the cylinder itself were cleared of any debris from previous usage, using acetone (Sigma-Aldrich) and a metal scraper. The piston was cleaned of polymer debris using a ¼ inch drill (Skill-Chicago) with a wire brush attachment instead of a drill bit. The cleaned ram was passed through the cylinder to test the smoothness of the inner wall. A smooth movement of the ram through the cylinder ensured the absence of debris. The 0.014 mm wire screen (Alex James, Greer SC) was replaced with a new one and fastened to the cylinder head die. Copper-based anti-seize lubricant (Fel-Pro Chemical Products) was spread delicately over the screws that attached the die to the cylinder, before fastening them. Resomer L210 resin was poured in the cylinder until it was 2/3rd full and then the ram was inserted. The ram was then used to press the piston into the cylinder, employing the hydraulic press (Central Hydraulics, Taiwan) so that the loose resin was converted to a tightly packed pellet with removal of air from the particle interstices. The free circular movement of the ram in the cylinder was tested after pressing. A graphite sealant (SEPCO, Pelham AL) was placed around the ram before further packing with a bronze sealing screw, which was fixed on top of the ram and the cylinder assembly. During tightening of the screw, care was taken to ensure that there was free circular motion of the piston, with periodic checking of the ram movement. The tightening of the screws was stopped just before this rotary movement ceased. The loaded cylinder-piston combination was placed in the vertical position, within the heating jacket of the extruder.

The jacket was then shut tightly, after inserting an external thermocouple. A heating current was applied to the conducting wires of the heating jacket in order to melt the polymer. The set-point of the heater was fixed at 190 °C. After approximately 45 minutes of heating at this rate, downward movement of the piston, without any application of the force, was typically noticed, indicating polymer melting, thus allowing the extrudate to pass the spinneret die.

The ram was then driven by an actuator (Joyce-Dayton) that was adjusted to a speed of 1.3 to 1.6 cm/sec. The extruded, molten polymer was cooled by a blower that was built into the extrusion apparatus. The diameter of the crude extruded polymer fiber was measured to be 0.25 ± 0.2 mm. The amorphous state of the polymer was suggested by its clear appearance; its high brittleness supported the observation. Thus the un-drawn fiber was wound with extreme caution on a 60 mm cardboard bobbin.

2.1.3 Fiber Drawing Methodology

The polymer extrudate couldn't be used as is, since it was brittle and thick. It needed to be drawn to a nominal fiber diameter of 0.10 mm, which required a drawing ratio of 1:5 or 1:6. Owing to the brittleness of the fiber, a manual drawing procedure was implemented for this purpose. A hot plate (Fisher Scientific) was set at 55 deg C, which is the published glass transition temperature of PLLA. The plate was covered with aluminum foil and was cleaned with 70% Ethanol Solution to maintain fiber cleanliness. Using a Vernier calipers the extruded fiber was measured and the diameter recorded; as noted, nominally, 0.25 mm. One end of the fiber was attached to the hot plate using tape, and the other end was held by tweezers. After visually confirming the visco-elastic state of the fiber, one end was slowly pulled till the color of the fiber changed from translucent to white, signifying the transition of the fiber to the crystalline state. The diameter of the drawn fiber was measured for uniformity, nominally 0.10 ± 0.02 mm. It was stored in a desiccator until further need.

2.2 Design of PLLA-based Biodegradable Stents:

Dr Su et al designed the "outer coil" stent in the lab at UT-Southwestern Medical Center [1]. During the latter part of his work it was realized that the outer peripheral coils of the design would be disposed to scour the vessel wall and that relatively high pressure are required to attain full deployment. He had suggested the design of an "inner coil" stent that was later modified and developed by a succeeding student, Pankaj Satasiya, also at UT-Southwestern [2]. Mr. Satasiya was able to obtain a single ply inner coil stent that removed the threat of wall scouring, and required lower pressures for the full deployment. Still further improvements were developed in the MS Thesis of Sushma Gampa [3,61]. Most details of that work pertain to an alternative two-ply design outside the scope of this investigation; however there are several useful alternative winding and glue-point strategies relevant to this work.

Fabrication of this inner coil stent required five principal operations:

1) Extrusion and drawing of PLLA fiber, as described.
2) Manual winding, in continuous fashion, of the fiber around preset, co-parallel mandrels, arranged on a metal jig.
3) Weaving of separate PLLA fibers of the same size, along the coil and co-parallel with the mandrels

4) Gluing of these “longitudinal” fibers to the coil fibers.
5) Termination of the longitudinal fibers and trimming of loose ends.

Extrusion of the PLLA fiber from scratch has been detailed in the above section.

2.2.1 Manual winding of the Fibers on Jig-mounted mandrels:

The selected inner coil stent design has 3 internal loops, with longitudinal fibers for reinforcement. The polymer fiber is wound around mandrels to form these loops, following a particular pattern. Stainless steel rods (Small Parts Inc, Miami FL) were used as mandrels. Three rods of 0.35 mm diameter and a central rod of 0.63 mm diameter were mounted on a jig designed by Gampa [3,61] to yield a 1.5 mm diameter furled stent (Bio-Instrumentation Lab, UTSW) as shown in Figure 2.1.

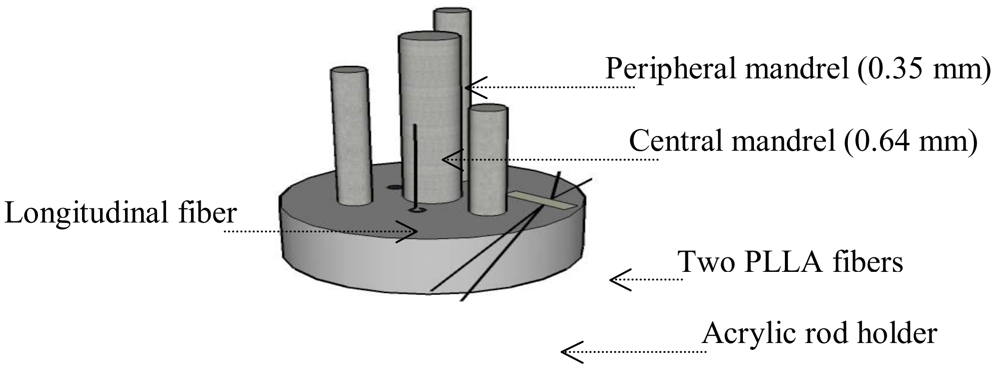


Figure 2.1 Rod holders with the arranged fibers and mandrels. Adapted from Gampa [3].

As shown by Su, Satasiya, Gampa and others, and vouched for in this author’s experience, stent winding requires very careful technique. The stent quality was determined by many, seemingly small factors, such as the distribution of fiber stress within the stent, absence of torsional load, integrity of the wound fiber and most of all, the local diameter of the stent. The method needs extreme caution and high practice and is highly prone to errors if not done carefully.

The current winding process starts with the placement of the central rod and the peripheral rods within the appropriate holes on the jig and taping them together at the bottom [61]. Care must be taken that sufficient fiber length is available on the top of the jig to fabricate a stent of the desired length. PLLA fiber of sufficient length was cleaned with alcohol swipes. One end of the PLLA fiber is taped at the bottom of the jig after looping it through the central mandrel with the other end remaining free for winding around the peripheral mandrels. Three pieces of identical PLLA fiber, each of 5 cm length, were wiped with 70% ethanol and inserted into the small clearance holes for longitudinal fibers on the rod holder. These act as reinforcement fibers to provide additional mechanical strength to the stent and to maintain integrity of the stent. The next step involves looping the fiber around each peripheral mandrel and under the subsequent longitudinal reinforcement fiber, thus returning to the starting point**.** This first coil turn consisting of 3 loops should serve as a benchmark to maintain the pitch of all subsequent loops as the fabrication of the stent proceeds. The next step involves looping each fiber on each peripheral mandrel and over the longitudinal fibers**.** In short, the longitudinal fibers were arranged in an alternating pattern, being kept outside the coil for one turn and inside the coil for the next turn, and so forth. This over and under design has to be procedure was followed for a total of 15 rotations to make a stent with a 15 mm length. The termination of the stent has to be with the fiber over the reinforcement fibers

2.2.2 Gluing of the Reinforcement Fibers

The fabrication of a 15 mm inner coil stent with a over and under design is described in the previous section. However if the stent is taken off the mandrel at this point, it will disorganize completely as the essential factor that will maintain the reinforcement fibers and the helical fiber in their respective places is absent. This procedure is the “gluing” of the reinforcement fibers to the peripheral loops.

2.2.3 Materials and Methods

Two gm of L-210 resin (Bohringer Ingelheim, Germany) is dissolved overnight in 10 ml of chloroform (Sigma-Aldrich, St. Louis, MO). Using a burner, 0.5 mm diameter, sharp-ended glass capillaries (Drummond Scientific) are prepared. The capillaries are placed in the glue overnight for loading by wick action. The glue-filled capillaries are inserted into a pin at the tip of a Micro Injector II (Drummond Scientific).

The primary condition for accurate attachment of the longitudinal fibers to the helical fiber is that the two fibers should be securely touching each other at the attachment point. Su and Satasiya defined a peculiar method of gluing of the reinforcement fibers. They followed a “fillet system” in which glue drops were delivered as shown in Figure 2.2.

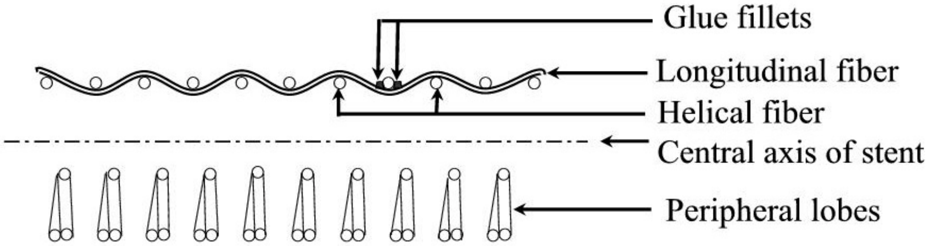


Figure 2.2. Median and transverse planes of the single ply inner-coil stent, noting typical glue fillet placement sites.

Figure 2.2 depicts the typical gluing methodology adopted by previous students (although there were variations in selected circumstances). This general method is extremely tedious; it requires consideration of many factors, such as fiber and stent size, microscope capabilities and the tortuous nature of the threaded longitudinal fibers.

In my experience, a gap is sometimes created between the helical coil and the longitudinal fibers**.** This gap is extremely difficult to spot under the microscope and hence when applying the glue fillet to what is perceived as a “gluing spot” the weld fillet may not have been properly applied, resulting in the “snapping” of the reinforcement fibers from the helical coil during stent deployment (expansion). A second important observation is that the glue solvent (chloroform) dissolves the surfaces of adjoining longitudinal and circumferential fibers, making the fibers as well as the weld weak and prone to damage during deployment. An alternative to the “spot fillet” technique is to deliver the glue over a longer portion of the circumferential fiber (shown in cross section in Fig 2.2)**.** This has certain advantages, the first being the broader spreading of the glue ensuring proper gluing of the reinforcement fibers to the helical coil. Secondly, the greater surface area provides for faster evaporation of chloroform, thus reducing fiber erosion. One critical observation: Even if the glue does partially dissolve the fiber, the residual PLLA in the glue still holds the weld together under deployment pressure, which is not seen in the "spot fillet" method. The most important feature of this broader fillet coating technique is the improved control of the glue site. The extremely tight spots where glue needs to be delivered are more reliably covered in this fashion. This is especially important, since the entire process of stent fabrication is manual.

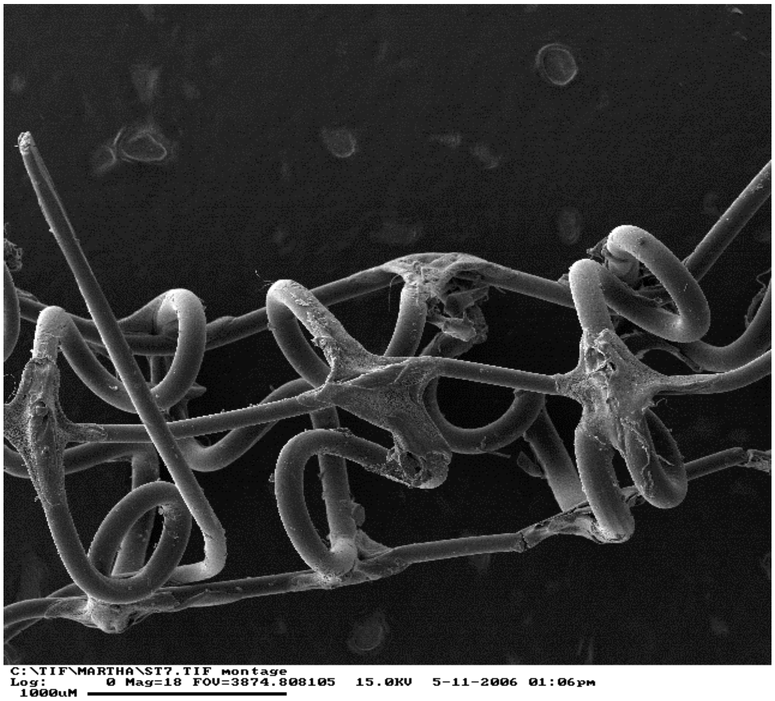


Figure 2.3 SEM view of typical stent gluing sites.

2.2.4 Termination of the Fibers and Trimming Loose Ends

Once the stent is fabricated the ends need to be terminated to ensure "locking" of the reinforcement fiber. The analysis of a inner coil stent will have a single helical coils supported by 3 longitudinal fibers all throughout the stent except the ends. The ends have only one longitudinal fiber for support. The angioplasty balloon has been designed to inflate at the ends first and then in the middle. This peculiarity will expose during expansion of the stent, both ends to greater stress from the inflating balloon, which as said previously first expands at the stent ends, later expanding the middle regions. Hence increasing the stress bearing capacity of the end became a critical point in the design. A procedure executed at both the ends of the fibers required the nearest longitudinal fiber from the desired endpoint of the helical fiber to be directed in a U-turn to pass underneath the first coil, above the second coil and then again underneath the third coil. Following this the points were glued carefully. Satasiya [2] and Gampa [61] described this exacting termination process in high detail.

2.2.5 Precautions During the Stent Fabrication Process

The stent fabrication process described the preceding passages necessitates certain precautions which critically determine the quality of the finished stent. During fabrication, the tension distribution in the fibers is one critical factor. Right from the start, care has to be exhibited during the winding process: Similar amounts of force must be applied when pulling the fibers around the mandrels. If this is not done, then convergence of the mandrels occurs towards the top (distal) end of the furled stent. Nonuniform tension develops cumulatively as the helical winding proceeds, resulting in a conical shape rather than the desired cylindrical shape. To partially remedy this, small pins can be inserted between the mandrels to maintain gap tolerances. Tension distribution still cannot be even in this manual winding process, but the force distribution is improved, and results in “more cylindrical” unfurled stent. The fiber diameter should be measured at discrete points to ensure uniformity. The fiber can snap during winding as nonuniform force is applied; the effect of the chloroform weld solvent on a relatively thin fiber (more damaging compared to thick fibers) is a related consideration. As far as possible, the trimming of extra fiber at completion of winding should be done whilst the stent is on the mandrel; this helps to prevent cutting the wrong fibers. The figure describes a detailed explanation of how a furled and a unfurled stent should look once complete and deployed.

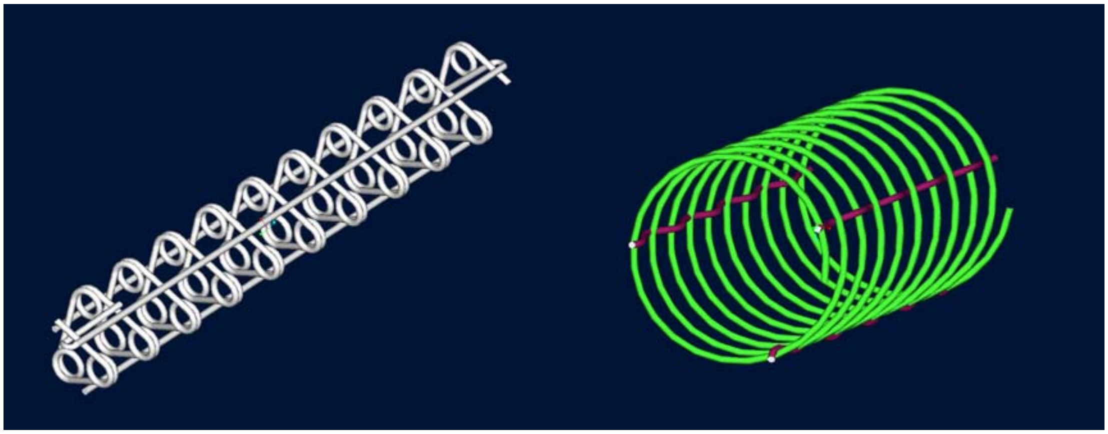


Figure 2.4 Schematic views of furled (left) and expanded (right) single ply stent. With permission (Satasiya thesis [2]).

The initial and final diameters of the stent depend on the diameters of the central and peripheral mandrels, the number of peripheral mandrels and the peripheral mandrel circle diameter. Based on these features the stent expansion is defined as:

$$\%\ \text{Expansion} = \frac{\underline{(\text{Final Diameter} - \text{Furled Diameter})}}{\text{Furled Diameter}} * 100$$

The measurements of the stents were done at three different points, which included both ends and the middle. These values were averaged and reported as the final diameter of the furled stent. However this technique is employed, there remains a quality control, repeatability issue. As the entire process is manual there is a large potential variation from one stent to the next. The fabrication techniques were exhausitvely refined in order to maintain the expanded stent diameters in the range of 3.0 ± 0.25 mm. In my experience, the resulting stent properties would provide fair results during the experiments.

2.3 Deployment of Stents With the Balloon Catheter

Once the completed furled stent was taken off the mandrel then it needed to be deployed in order to check the aforementioned unfurled diameter.

2.3.1 Materials and Methods

Two vendors' balloon catheters were used for stent expansion: the 3.5 x 15 mm balloon catheter, courtesy of Dr. Su (Biosensors International, Irvine CA) and the 3.0 x 30 mm Ninja™ balloon catheter (Cordis Corporation, Warren NJ). The Basix 25 syringe pump (Merit Medical Corp., Salt Lake City UT) was used for balloon pressurization.

The stents were mounted with extreme care on the balloon catheter that had been deflated with partial vacuum. A guide wire was employed, placed in the catheter central channel and extended along the axis of the furled stent, to assist in gently slipping the stent over the furled balloon without disturbing the integrity of the stent. Using the syringe pump, pressures of 12 atmospheres were generated in monotonic fashion, with the time periods for full pressurization varying from 5 seconds to 2 minutes. Following completion of the expansion, the pressure was released and the expanded stent was taken off the balloon catheter and measured using the digital Vernier scale.

2.4 Removal of Kinks Formed During Expansion of the Stent:

As noted in the original pictures, the Su et al stent deployed more towards a triangular configuration rather than a circular configuration. Furthermore, it was also noted that the helical fibers tended to kink. It was recognized early on that the fibers are exposed to a 360 degree rotation during stent expansion; since the fibers are constrained this results in a torsional shift, producing kinks resulting in stents that appear as shown in Figure 2.3.

2.4.1 Materials and Methods

Thirty stents were fabricated by the technique described above. Water was heated to temperatures ranging from 37 to 55 Deg C in an oven, with periodic measurements to ensure the stability of the water temperature. The Ninja^{TM} 3.5 x 30 mm balloon catheter

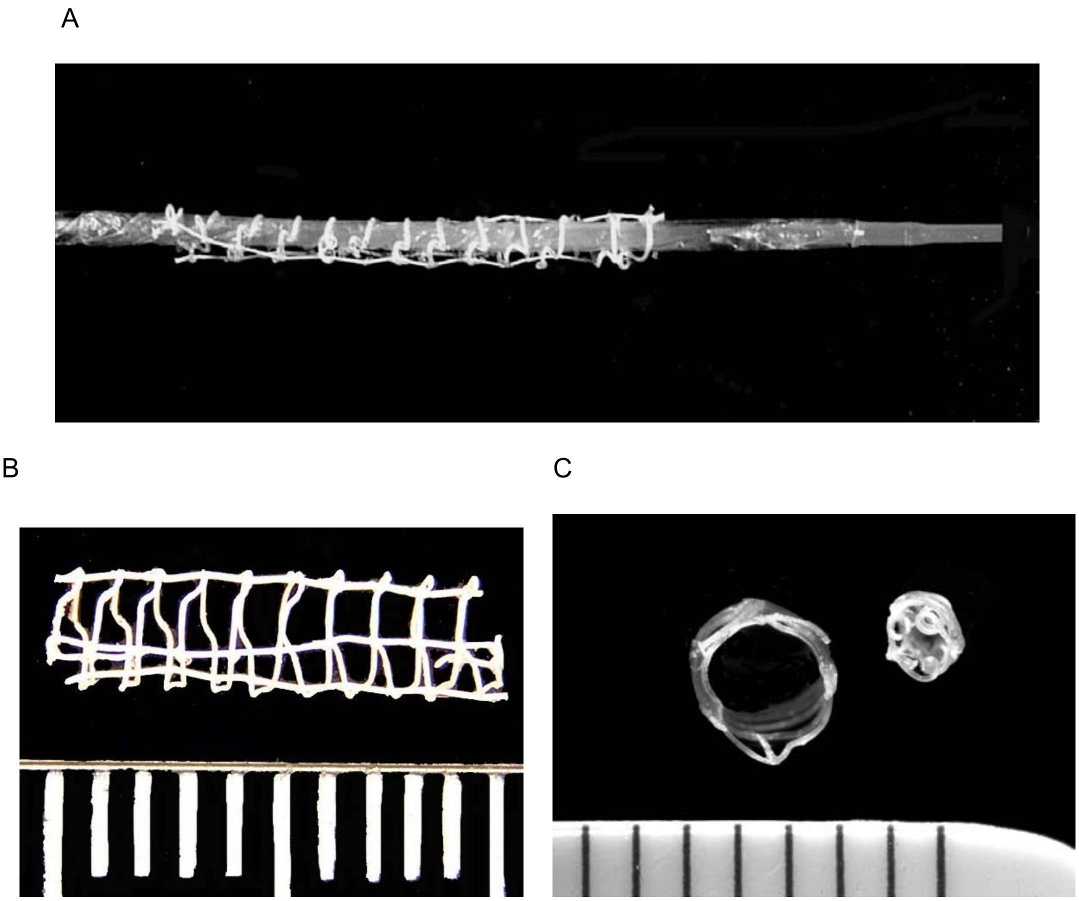


Figure 2.5 Views of stent expansion, with mm ruler markings. (A) Furled stent mounted on the balloon; (B) Longitudinal view of the expanded stent with mm ruler markings; (C) Cross-section of an expanded stent (left) and a furled stent (right).
Reproduced with permission.

(Cordis Corporation, Warren NJ) and the Basix 25 inflation device (Merit Medical Corp., Salt Lake City UT) were used for stent expansion.

The heated water was poured in a glass beaker that was set on a heating plate, controlled to the desired temperature by periodic measurements using a thermometer, to ensure maintenance of the preset temperature. Six stents were used for the experiment. After placing the stent on a balloon catheter the syringe pump was filled with the heated water. The balloon catheter was inserted into the beaker and taped to the edge of the beaker. Pressure was slowly increased and held at the target pressures for 45 seconds, after which the pressure was released and the stents diameter was measured as described previously. Similar procedures were performed over a range of temperatures between 37 and 55 Deg C. Pictures of the deployed stents were taken, and images were observed for kinks, recoil and shape change, which were recorded.

2.5 Glue Testing and Design

As described previously, PLLA based glue was used by Su to attach the struts to the helical coil. PLLA glue has very strong adhesion and good mechanical strength that was evident during stent deployment at higher pressures. The chloroform content of this PLLA-based glue did not evaporate quickly, leading to considerable damage to the fibers and resulting in many defects such as breaking of the fibers during the fabrication process, or during deployment. Thus a search for a alternative to PLLA glue was defined.

2.5.1 Materials and Methods

A total of six poly-lactic acid homo-polymers and co-polymers were used for this purpose. Each polymer was weighed out in specific amounts and a measured quantity of chloroform was added to dissolve the polymer. Table 2.1 describes the polymer viscosity, percentile mixtures of co polymer content along with the quantity of chloroform used to prepare glue. Thirty two stents were fabricated based on the procedures described earlier. The Ninja^{TM} 3.0mm x 30 mm balloon catheter (Cordis Corporation, Warren NJ) and Basix 25 inflation device (Merit Medical Corp., Salt Lake City UT) were used for stent expansion.

Table 2.1 Glue types

No	Polymer	Company	Chloroform Content
1	PLLA	Boehringer Ingelheim	10 ml
2	PDLLA	Boehringer Ingelheim	10 ml
3	PCL	Boehringer Ingelheim	10 ml
4	PLGA	Boehringer Ingelheim	10 ml
5	PDLLA-PLLA (20:80)	Boehringer Ingelheim	10 ml
6	PLLA-co-PDLLA (50:50)	Boehringer Ingelheim	10 ml
7	PCL-PLLA (80:20)	Boehringer Ingelheim	10 ml

In batches of 4, all reinforcement fibers were glued specifically with the particular glue type and deployed. All observations during gluing and post gluing were noted down and termed as the characteristics of the glue type.

2.6 Melt Temperature of Curcumin

Curcumin (anti-proliferative agent) was the drug used to load the fibers. The melt temperature of curcumin was checked, as described in the previous sections for the melting temperature of HMW PLLA.

2.6.1 Materials and Methods

Curcumin (Sigma Aldrich, MW 368.4) was used along with a High Temperature Oven (Thermolyne, Iowa). Curcumin samples, 0.3 gm each, were placed on aluminum foil and exposed to a range of temperatures from 150 to 190 Deg C. The sample exposure time to the elevated temperature was a maximum of 4 minutes. Observation of the coloration of the curcumin after melting was observed and noted down.

2.6.2 Curcumin Loaded PLLA Blend Preparation

Three more batches were made by the process of addition of curcumin in the solvent allowing good mixing as described above. Curcumin was added in 5 % by weight to the solution. In order to avoid clumping of curcumin aggregates, the rate of solvent evaporation was controlled (Dr. Su, personal communication). This was done by covering the Petri dishes with aluminum foil decorated with small punch holes to allow controlled escape of the chloroform solvent. Films of good consistency and uniformity were obtained by this means.

2.7 Extruding PLLA Fiber with Curcumin

Su et al had successfully loaded curcumin into low molecular weight PLLA (137 KD) and performed various experiments, including inflammatory tests of fiber samples, employing Dr. Tang's method [1]. However curcumin-loaded stents were not fabricated for these experiments. Melt extrusion of curcumin in high molecular weight PLLA, typically Resomer L-210, was attempted by Gampa in our lab; however the results were unsuccessful. This thesis explores the loading curcumin into lower MW polymers. Hence the process of melt extrusion of curcumin in HMW PLLA remains unexplored.

2.7.1 Materials and Methods

Ten gm of Resomer L-210 (Boehringer Ingelheim, Germany) was measured and stored in a bottle. Curcumin, 5 % by Resomer weight was carefully measured and stored separately. The batch extruder (Alex James, Greer SC) was prepared for extrusion as described previously [1,2]. A kitchen blender (Magix, Chicago IL) was cleaned with 70 % ethanol prior to use.

The Resomer and curcumin aliquots were poured in the blender, which was then set to the slowest speed. The mixture was removed after 30 minutes of blending. All curcumin particles adherent to the sides of the blender were brushed into the comminuted mixture of PLLA and curcumin. These mixtures were stored in a bottle until and transfer to the cylinder of the batch extruder. The extrusion process described earlier was altered in order to successfully extrude curcumin-loaded fibers. The previous sections describe the extruder temperature set for pure PLLA (Resomer L-210), i.e., 190 Deg C. However it was necessary to set the PLLA-curcumin pellet to a lower temperature, 187 Deg C. It is critical in this case that as soon as the melt is noticed without any force applied to the piston, the actuator needs to be set at a speed of 0.9-1.0 cm/min to ensure constant pressure application on the melted material. This highly viscous material does not exhibit a free flow nature like pure L-210, and hence continual application of pressure is important. The extruded fiber is then cooled down and stored in a desiccator until further use.

2.8 Deployment and Collapse Pressure Tests for Curcumin-loaded Stents

Twelve stents were prepared using curcumin loaded extruded and drawn fibers having an average diameter of 0.10 ± 0.03 mm. The stents were then deployed using a Ninja 3.0 x 30 mm balloon catheter (Cordis Corporation, Warren NJ). The initial and final diameters were checked using the Vernier caliper. The incremental diameter change after each 1 atm increase in pressure was noted down till the stent was fully deployed.

Four batches of 3 stents each were then incubated in PBS solution for 4 weeks at a controlled temperature of 37 Deg C in a incubator. Each week, the PBS was replaced with fresh PBS. At the end of each week one batch of curcumin-loaded stents was taken out and dried in air for 1 day. Then after carefully cleaning them of remnants of dried PBS salts they were subjected to collapse pressure tests.

2.9 Recoil Tests for Curcumin-loaded Stents

Nine stents were prepared using the curcumin-loaded fibers. The initial and final diameters of the stents were noted down. In batches of 3, the stents were immersed in PBS solution for 4 weeks at a controlled temperature of 37 Deg C in an incubator. Each week the PBS in the flask was replaced with fresh PBS. At the end of each week the stents were taken out and the diameters were remeasured. The percentage recoil was calculated using the formula:

$$\%\ \text{Recoil} = \frac{[(\text{diameter on inflated balloon})-(\text{diameter on deflated balloon})]}{(\text{Diameter on inflated balloon})} * 100 \tag{2.2}$$

2.10 Development of a Mini Batch Extruder

Polymers are expensive materials to purchase. Innovative fiber technology is always a focus of these stent studies. The Alex James extruder was incapable of extruding small quantities of material. A minimum of 6 grams was needed in order to get it to function properly. Thus the need arose for design of an extruder that could be used with small quantities of polymer and yet could extrude long lengths of test fiber.

2.10.1 Design of the Mini Batch Extruder

The design of the mini-extruder was based on a simple cylinder and ram design. Any heated (melted) element with the right application of pressure can be forced out of a small bore is the main guiding principle on which the technology was developed.

2.10.2 Materials and Methods:

Tool steel (Grade A) was used for the cylinder, ram and end plate. This metal exhibited the property of becoming harder with application of heat each time (personal communication, Physics Workshop personnel). A 15 mm diameter hole was drilled coaxially in a 60 mm diameter x 45 mm height cylinder. The surface of the bore was polished for a smooth finish. The diameter of the ram was 14.95 mm controlling the tolerance to a minimum of 0.05 mm. Tolerances were controlled ad hoc to obtain a good fit for the ram, yet allow slow, easy movement of the ram without any application of force. An end plate was counter-bored to fit the cylinder outer diameter was constructed, with a 0.2 mm bore in the center serving as extruder die. A good fit between the end plate and the cylinder was ensured, and attachment was by means of ¼ inch machine screws. The design was intended to accommodate 1 gram of polymer inside the extruder and process about 10 feet of fiber of 0.2 mm thickness. A silicone-coated heating tape (Cole-Palmer) was wrapped around the cylinder-ram assembly to serve as the heating element. It was powered by a variac: the system was calibrated with a digital thermometer placed on the cylinder wall inside the heating tape. The first trial was done with L-210 0.5 gm and curcumin 5 wt. %, that were mixed thoroughly with a spatula and poured in the cylinder. The temperature of the heating tape was set to 190 Deg C. Three lead bricks, each weighing 20 lb, were used for application of pressure on the ram. As soon as movement of the ram was seen, without application of force, indicating melting of the polymer, one lead brick was placed on top of the ram. Bricks were added consistently until the entire polymer batch was extruded.

2.11 Preparation of Blended Polymer Fibers:

It is well documented that: 1) PLLA induces host inflammatory responses; 2) its brittle nature appears during the degradation process; degradation is a relatively slow process. To improve on these adverse characteristics for stent fibers, random and block co-polymerizations of lactide with other monomers such as ε-caprolactone (CL) and cyclic carbonates (CC) were performed, using tin and rare earth metal complex catalysts [45]. The degradation rates of these copolymers were favorably increased. Such polymer blends may provide other characteristics that favorably veer away from the original polymer properties. Thus polymer blending can be used to optimize mechanical properties, i.e. modulus, strength, crystallinity and perhaps also improve biocompatibility. Popular blends recently studied for subsequent inflammatory responses include PCL-PLLA and PLGA blends [45]. Su noted that since curcumin has important anti-inflammatory characteristics, it might serve to reduce the inflammatory potential of these bioresorbable aliphatic polyester polymer blends. Accordingly, four types of blends were developed to assist in the development of stent material.

2.11.1 Materials and Methods

Low molecular weight PLLA (L-207, LMW-PLLA) and high molecular weight PLLA (L-210, HMW-PLLA) and poly (D,L lactide) (L-202, PDLLA), all from

Boehringer Ingelheim, Germany were specifically ordered for blend development. Curcumin (Sigma-Aldrich, St. Louis MO) and chloroform (Sigma-Aldrich) were used as drug and solvent, respectively. Ten Petri-dishes from Fisher Scientific and a stir plate (Thermolyne,Iowa) were selected for use. The mini-batch extruder (section 2.10) was prepared for extrusion of the blends to develop fibers.

Three PLLA blends were prepared, each varying in the content of LMW- PLLA and HMW-PLLA. Two blends of PLLA:PDLLA were prepared, using both HMW-PLLA and LMW-PLLA. All five blends were prepared without the impregnation of curcumin at first. A second batch of blends was prepared that were loaded with curcumin throughout the polymer fiber by melt extrusion. HMW-PLLA (referred to as L-210 hence) and LMW-PLLA (referred to as L-207 hence) were weighed out in ratios of 60:40, 40:60 and 50:50. Chloroform, 300 ml, was added as a solvent. Each mixture was poured in a glass bottle and a magnetic metal stirrer was added and the mixture stirred overnight to allowing L-210 and L-207 to dissolve completely. Solutions were carefully removed using a glass pipette and placed in measured quantities on a Petri dish and left in a vacuum hood overnight for solvent evaporation. These films were then transferred to a temperature-controlled oven and set at 50 Deg C overnight to remove any remnants of solvent. After carefully removing the films from the dishes these films were cut into relatively large pieces. They were then transferred to the cylinder and pressed into a pellet. The large pieces avoid them from sticking to the side of the cylinder and restrict the movement of the ram. The fibers were extruded at a melt temperature of 180 Deg C.

2.12 Blending PDLLA-PLLA:

PDLLA-PLLA blends were also made in the same manner explained above and extruded to develop fibers. However the temperatures maintained for extrusion of PDLLA-L210 were at 170 Deg C and PDLLA-L-207 was done at 145 Deg C. These temperatures were obtained by cutting small pieces of the films and setting them at controlled temperatures in a oven, ranging from 145 to 190 Deg C. Observations were noted and an optimum temperature range was seen at which PDLLA-L210 melts; the lower end of the range was used as bench mark melt temperature for PDLLA-PLLA blending. A similar method was implemented for the PDLLA-L207 blend.

2.13 Blend Degradation Tests

The primary function of a bioresorbable stent is to provide mechanical support to the vessel until the vessel regains its strength. This is done by slow degradation of the bioresorbable stent fiber. The degradation occurs relatively slowly along with the loss of mechanical strength. The blended polymers described in section needed to be tested for degradation to get a fair judgment of the time frame before which the stent looses the radial collapse pressure withstanding capacity.

2.13.1 Materials and Methods

A 37 Deg C incubator with controlled humidity was used for this purpose. Blended PLLA fibers drawn to 1:4, 1:5 and 1:6. PBS solution prepared as per ASTM F 1635-95 (Re-approved 2000). These elements were used to study the degradation of blended polymers of the following contents: HMW-PLLA:LMW-PDLLA (50:50);

LMW-PLLA:LMW- PDLLA (50:50); HMW-PLLA:LMW-PLLA (60:40); HMW-PLLA:LMW-PLLA (40:60).

Fibers were drawn to the prescribed ratios and measured using an analytical balance before immersion in PBS. Weights were measured to 0.0001 ± 0.00002 gm. The fibers first were cleaned with 70 % ethanol wipes to remove any debris and dust. After measurement they were immersed in 5 ml PBS. The pH of the PBS at the start of the experiment was noted down. Twenty such samples were marked and placed in the incubator. After each of weeks 1 through 4, the fibers were taken out and dried at 37 Deg C overnight to completely remove the PBS. Once again they were wiped with a dry tissue to remove any clinging salt, then weighed. The pH of the end of week 1 to 4 was noted down. During the experiment the old PBS was replaced with 5 ml of fresh PBS every week.

2.14 Collapse Pressure Tests for PDLLA-PLLA Blended Stents

Any stent, polymer-based or metal-based, has the basic function of withstanding radial pressures exerted within the vessel wall. The stent collapse pressure is typically measured as a means to define this property. This was measured in a pressure chamber designed in our lab, manufactured by the Bio-Instrumentation Laboratory of UT Southwestern and used in all previous thesis and dissertation research in our lab [1, 2, 54].

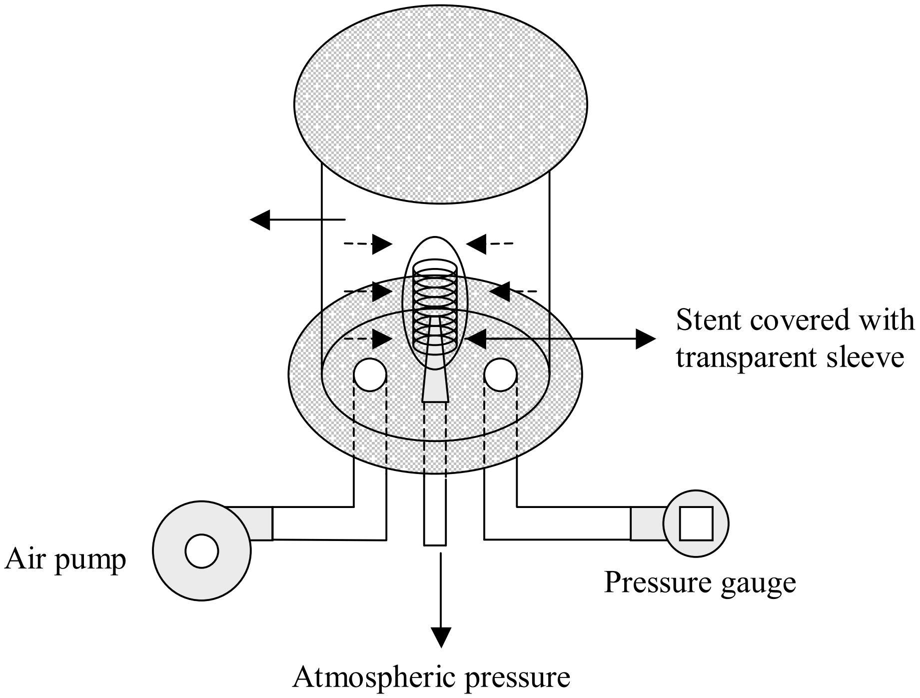


Figure 2.7 Compression chamber and the radial compressive forces on the stent

2.14.1 Methods

Stents were prepared by the methods described in previous sections. The collapse pressure tests were performed as described previously [1,2,54]. A fully expanded stent was mounted on the rubber tip of a nozzle, which is open to atmosphere. The stent was covered with a thin, transparent plastic sheath, which was sealed by a ligature at the base of the rubber tip. The pressure inside the chamber was increased by continuously pumping air with an air-pump (Microflex, Cole-Parmer Instruments Inc., Chicago, IL). This process continued till there was an observed change in the geometry of the stent. The pressure was released till the stent re-structured to its original configuration. This method was repeated till the stent was unable to recover completely. This was sustained as the collapse pressure of the stent.

2.14.2 Collapse Pressures Tests after Incubation in Saline For 4 Weeks

The slow degradation of the stents made from blends lead to a loss of the mechanical strength in time. In this procedure the 16 stents were made from different polymer blends. Four stents each were deployed and stored in PBS solution for 4 weeks. Each week the old PBS was replaced with fresh PBS. The method mentioned above was repeated on all 16 stents and collapse pressures were marked down for each stent made from the blends described above.

2.15 Stent Recoil Tests

There is a great deal of literature on the elastic recoil factors of various stents. There is minimal elastic recoil of earlier metal stent designs. However as metal stent filaments have become thinner, the elastic recoil comes into question. In contrast, polymer-based stents have more significant changes in the diameter due to elastic recoil which, as shown by Su et al, can lead to implant failures.

2.15.1 Materials and Methods:

Twelve stents were made for testing the recoil parameters. Three stents each were fabricated with fibers made from different blends. The stents were deployed fully using a Biosensors 3.5 x 15 mm balloon catheter. While on the catheter the diameter of the expanded stent was noted down at three different places. The stents were then immersed in PBS for two days and then once again the diameters were measured. The study was conducted for 2 days and the results were plotted.

2.16 Mechanical Tests on PDLLA-PLLA Based Stents

Satasiya [2] et al during his graduate level work performed tensile tests on HMW-PLLA fibers after incubation in saline for a period of 120 days. Gampa [3, 61] performed mechanical testing on the LMW and HMW PLLA blends. The specific aim of the study is to test the mechanical properties of HMW PLLA and LMW PDLLA blends with different percentages of each polymer.

2.16.1 Materials and Methods

The mechanical properties of the drawn fibers were measured using a tensile testing apparatus (INSTRON 5848 Microtester) equipped with a 25N static load cell (Instron Corp., Canton MA). Five samples of each fiber were tested at room temperature in uniaxial tension at an extension rate of 1 mm/min.

Fibers made from blends of HMW-PLLA:LMW-PDLLA (60:40) and LMW-PLLA:LMW-PDLLA (40:60) were drawn 1:4 as described earlier, then placed between the hydraulic clamps, carefully, and slowly tightened using the fine adjustment setting on the Instron tensile testing machine such that the length between the clamps was approximately 1 inch. After setting up the software parameters, a constant strain rate at 1mm/min was applied until the fiber snapped. From the stress-strain curves the following parameters were extracted: the slope of the elastic region was (Young's modulus), the maximum strength (tensile strength), and the maximal strain at which the fiber broke (breaking strain).

2.17 Thermal Analysis of PDLLA-PLLA Blends

Thermal transition in polymers like the glass transition temperatures, crystallinity, melting temperatures are important features that can be determined using a Digital Scanning Calorimeter (DSC). DSC is guided by the principle of constant temperature being maintained between the reference and the sample furnace by changing and varying the amount of heat flow between the two. Since the heat flow is maintained, any thermal transition in the sample will be recorded and can be used to analyze the thermal property of the sample within.

2.17.1 Materials ad Methods

A Pyris 6 Diamond Differential Scanning Calorimeter (DSC, Perkin Elmer Instruments, Norwalk CT) was used to perform the thermal analysis of the blends. After the PLLA samples were weighed carefully on a microbalance (UMX2, Mettler-Toledo Inc, Columbus OH), they were placed into 50 µl aluminum pans and sealed using a 10 µl aluminum lid and crimper (Perkin-Elmer Instruments, Norwalk CT). Pyris 6 Diamond Software was used to control the DSC system and also to collect and analyze data.

The initial step involved the DSC being heated to 600 Deg C for 30 min and then cooled down to back to 30 Deg C. This method is repeatedly performed until a uniform heating path can be obtained. The aluminum pans are cleaned with 70 % ethanol wipes and air-dried before being loaded with the samples. Utmost care has to be taken to avoid any contamination by placing the pans in a scintillation vials before use. About 8 to 10 mg of the samples are prepared and then placed into the pans. For calibration purpose two empty pans are sealed and placed in the reference and sample furnaces and allowed to equilibrate at 30 deg C for 10 min before beginning the heating cycle. The two pans were heated from 30 deg C to 210 ^{o}C, maintained at 210 deg C for 2 minutes and then cooled back to 30 deg C at a temperature ramping rate of 15 deg C/min with Nitrogen as purging gas. The heat flow path obtained is recorded as the baseline. After the baseline was obtained each sample was tested using two scans each. During all the runs an empty pan was maintained in the reference furnace. Two scans are needed as the first scan is successful in removing the inconsistencies and the data from the second scan can be utilized for analysis after subtracting from the baseline. The glass transition temperature (T_g) and the melting point temperature (T_m) were determined for all the blend films from their respective DSC curves.

The degree of crystallinity was determined from the respective enthalpy of crystallization (ΔH_c) and the enthalpy of fusion (ΔH_f) using the relationship:

$$\% \text{ Crystallinity} = \frac{(\Delta H_f - \Delta H_c)}{\Delta H_f} \text{ x } 100 \tag{2.3}$$

where $\Delta H_f = 93.7$ J/g , the enthalpy of fusion of a 100 % pure, crystalline sample of PLLA [61].

CHAPTER 3

RESULTS

The bioresorbable stents developed by Su are described in his dissertation, with all the details in regards to fiber production, stent fabrication and drug loading technology within the stent [1]. The original stent has a coil density and length of 1 turn/mm and 10 mm, respectively. Stents of different lengths and coil densities are readily produced, as noted in the Satasiya thesis [2]. The furled diameter of the coronary artery stent is important in the sense that the lower the furled diameter, the better will be the navigation of the stent in the coronary artery tree especially in the vicinity of more stenotic lesions [2]. This can be said true as long as furled stent flexibility is unchanged and the stent diameter changes proportionally to the furled diameter. The entire fabrication process being manual, it is hard to confirm these assumptions; they are observed through personal experience and adeptness in the fabrication of the stent.

3.1 Glue Technology Studies

Glues were prepared from different polymers, blends and copolymers. They were applied to affix the longitudinal fiber struts to the stent fiber coils. The focus was to attain a maximum deployment pressure without any breakages, “snapping” and loosening of the struts from the longitudinal fibers. Maximum stent deployment pressures for the various glues are charted in Table 3.1. Different results were obtained, depending on the glue composition.

Table 3.1 Glue composition, maximum expansion pressure, quality of the glue fillet and wettability

Glue Composition	Maximum expansion pressure	Threads from fillet	Surface wettability
PLLA	8 atm	Moderate	Excellent
PDLLA	3 atm	High	Moderate
PCL	1 atm	Low	Very Poor
PLGA	5 atm	High	Poor
PDLLA-co-PLLA (20:80)	2 atm	Low	Excellent
PLLA-co-PDLLA (50:50)	5 atm	High	Good
PCL-PLLA (80:20)	2 atm	High	Very Poor

Pressure: Maximum deployment pressure without fiber or weld breakage.

Quality is defined by the number and size of "threads", e.g., glue filaments projecting from the glue fillet.

Wettability: The ability of the glue to spread around the fibers at cross-over points. See Figs. 3.1, 3.2 as examples.

Excellent: High deployment pressure (4-8 atm), suggesting high bond strength between axial and coil fibers. Low to negligible filament formation. High surface wetting.

Good: Moderate deployment pressure (3-6 atm). Small numbers of filaments projecting from the fillet (3-8 mm). High surface wetting.

Moderate: Low deployment pressure (3-4 atm), or stent failed overexpansion test as defined in section (2.14). High number of threads formed on fillet. Moderate surface wetting.

Poor: Stent failed expansion test. Very high thread formation. Low surface wetting.

Very poor: Loss of stent integrity at ≤ 1 atm. High thread formation. No, or negligible glue adhesion to fiber.

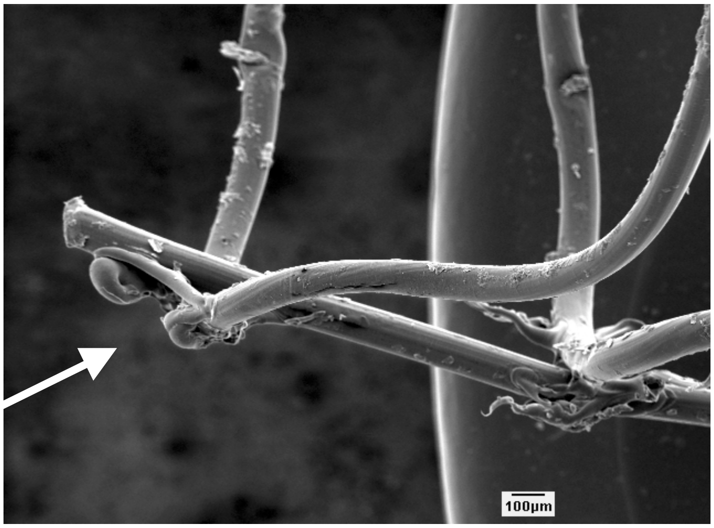


Figure 3.1 Poor wetting of glue to fiber surface.

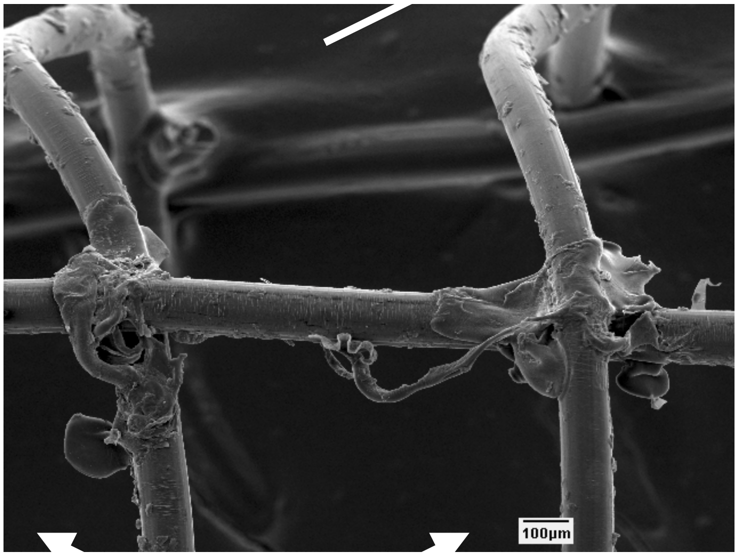


Figure 3.2 Moderate wetting of glue to fiber surface

3.2 Heat Treatment and Mechanical Annealing Effects

The pictures in Figure 3.3 show the different configurations of the stents exposed to different temperatures post-expansion, and the subsequent effects. Table 3.2 describes the pressures required for complete deployment, the time for which the pressures were retained and the visual observations of fiber kinking.

Table 3.2 Stent deployment pressure vs. balloon fluid temperature.

Temperature deg C	Pressure Atm	Time sec	Degree of kinking
37 - 42	4	35-45	High
42 - 45	4	35-45	Moderate
45 - 48	5	35-45	Low
48 - 52	5	35-50	Low
52 - 55	4	35-50	Low

Kinking of fibers during stent expansion is analyzed quantitatively by finite element methods in the MS Thesis of T. Welch [74]. As shown by Welch, the kinking effect is caused by plastic deformation of surface regions of inner coil fibers near the point of tangency of the inner coil with the main coil. Kinking at the site of tangency of the inner coil with the main coil was prominent when the fibers were expanded below 45 deg C and below 4 atm pressure. In contrast, kinking was significantly reduced when fibers were expanded above 45 deg C. The time for which the fully deployed stent was kept on the balloon under pressure varied between 35 to 50 seconds as noted in Figure 3.3 (B). For the 40 deg C expansion, the kinking effect was substantially reduced while for expansion temperature greater than 52 deg C it was completely eliminated. One can discuss a potential interdependency between the effect of temperature and pressure. Further analysis with different blends materials could prove otherwise and a relation between temperature and pressure could be noticed. It should be noted that during expansion of stents using nominal pressure rating (3-4 atm) for inflation with high temperature, we were able to provide for “kink free” stents. Hence we cannot be highly assertive about any interdependence between balloon pressure and fluid temperature

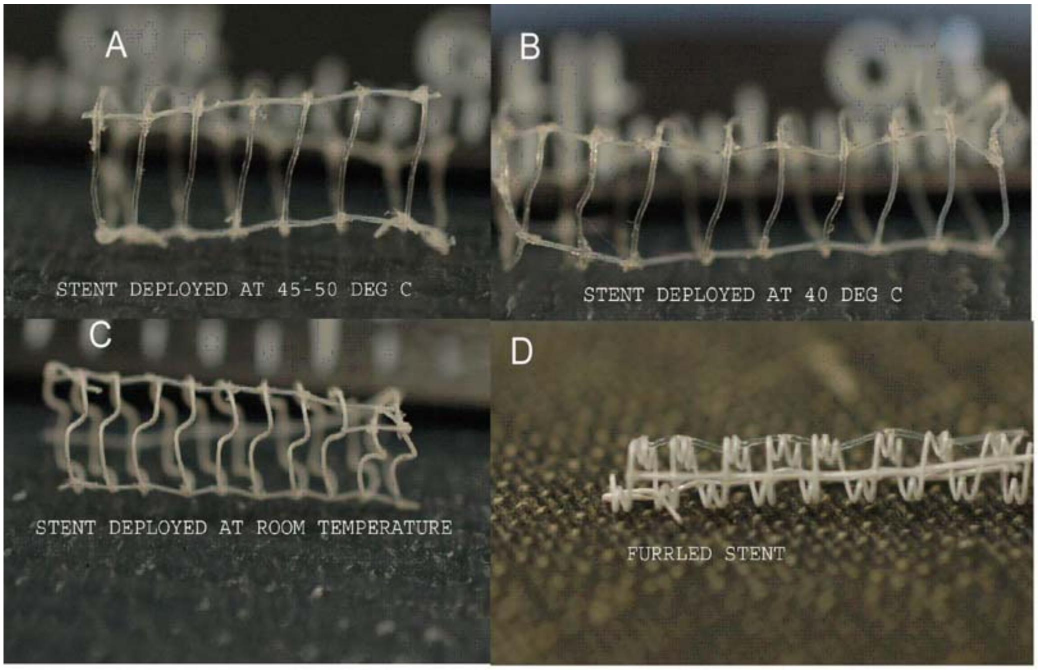


Figure 3.3 Stent deployments with saline-filled balloon catheters at various saline temperatures. Similar expansion pressures and times were employed in all cases. A) Deployed at 45-50Deg C. B) Deployed at 40 Dec C. C) Room Temp. D) Furled Stent

Even though the process successfully provided kink-free stents, this is not a recommended procedure for the clinical setting, mainly because temperatures as high as 50 deg C may have a thrombotic effect on blood flow, and the possibility of damage to the vessel wall initiated by lipid melting in cell membranes. Cells present within the blood stream may also be damaged by the same means.

3.3 Deployment Characteristics of Stents Made from Polymer Blends

3.3.1 Expansion Pressures of Blended Stents

The pressure required to expand a coronary artery stent is an important criterion for successful deployment. Stents that expand at lower inflation pressure will, in theory, have better apposition with the vascular wall. Higher deployment pressures will increase the likelihood of vascular injury, including the risk of plaque rupture. Figure 3.4 presents plots of the average stent diameter as a function of balloon pressure during inflation, obtained for stents made from different polymer blends. HMW-PLLA fiber stents in this series have an average deployment pressure of about 4.5 atm. The average inflation pressure required to expand a HMW-PLLA:LWM-PDLLA (50:50) stent to its nominal diameter (3.0 ± 0.2 mm) varies by 0.5 to 1 atm from the value required to inflate an HMW-PLLA-based stent. The pressure required for full expansion of the LMW-PDLLA: LMW-PLLA (50:50) stent is the lowest, requiring between 2.0 and 2.5 atm.

At times, inflation pressures of up to 6 atm were noted, but these are disregarded, as they could be erroneous results due to human error during the fabrication process. Some of the stents broke during inflation and the snaps were at the bonding point and could not be seen, and hence inflation pressure was increased.

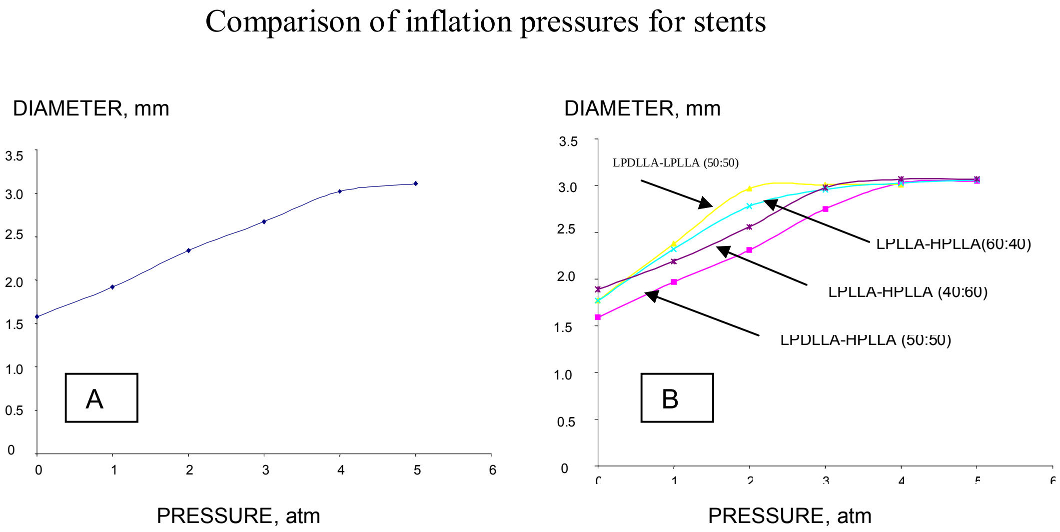


Figure 3.4 Inflation pressure profiles of PLLA and blended fiber stents.
A. PLLA, B. Fibers of various blends.

. Typically, 8 out of 10 fabricated stents will fall within a minimal deviation from the standard deployment pressure. However, breakage of the stent due to such factors as overexposure to the chloroform solvent in the glue, batch differences between glue compounds, and unequal distribution of tension during the winding of the stents on the mandrel (a critical factor) can occur. From personal experience, the tension distribution within the fibers seems more even towards the base of the mandrels; as winding progresses the tension distribution changes in order to accommodate the "curving" of the mandrels as shown in figure 3.5. It can be assertively said that the pressure required to reach full stent expansion varies inversely with increasing content of low molecular weight polymer within the blend (PDLLA or PLLA).

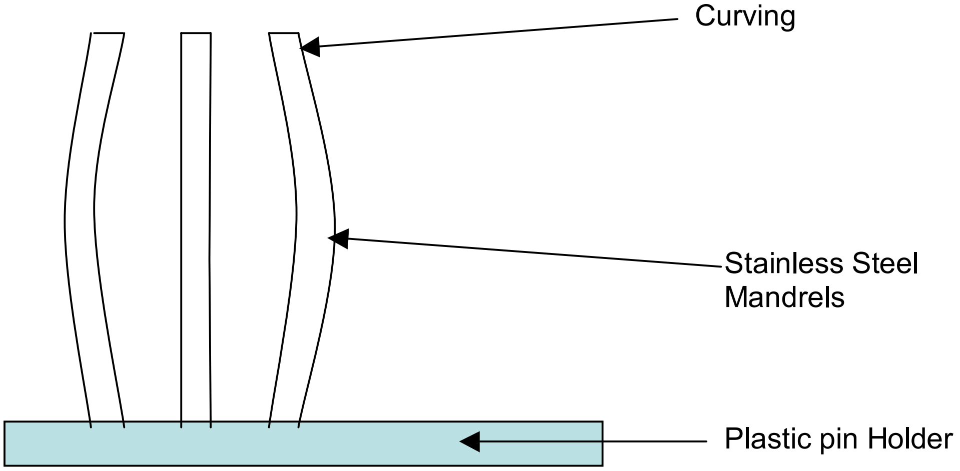


Figure 3.5 Indicator of curving of mandrels during stent fabrication

3.3.2 Recoil Properties of Stents Fabricated from Blended Fibers

The stents made from homo-polymers and blends were incubated at 37 deg C for 0, 12, 24, 48, 72, 96, 120 and 144 hour periods. The diameters before and after the incubation period for stents made from PLLA blends are shown in Figures 3.6. They all show a reduction in diameter, by 2-3 %, by the end of week one. Stents made from HMW PLLA-LMW PDLLA blends, when exposed to similar conditions, also showed recoil effects. Diameter shrinkage was observed within 5 minutes of balloon removal. However this diameter was not considered for initial calculations since there was large variation of diameters within all the stents that were taken off the balloons after achieving full inflation. Thus the diameters noted after 5 minutes from removal from the balloons were considered as initial diameters. At the end of one week of incubation for HMW PLLA-LMW PDLLA blends, 4-5 % recoil was observed. Stents made from LMW PLLA-HMW PDLLA blends showed a decrease of 8-10 % by the end of week one.

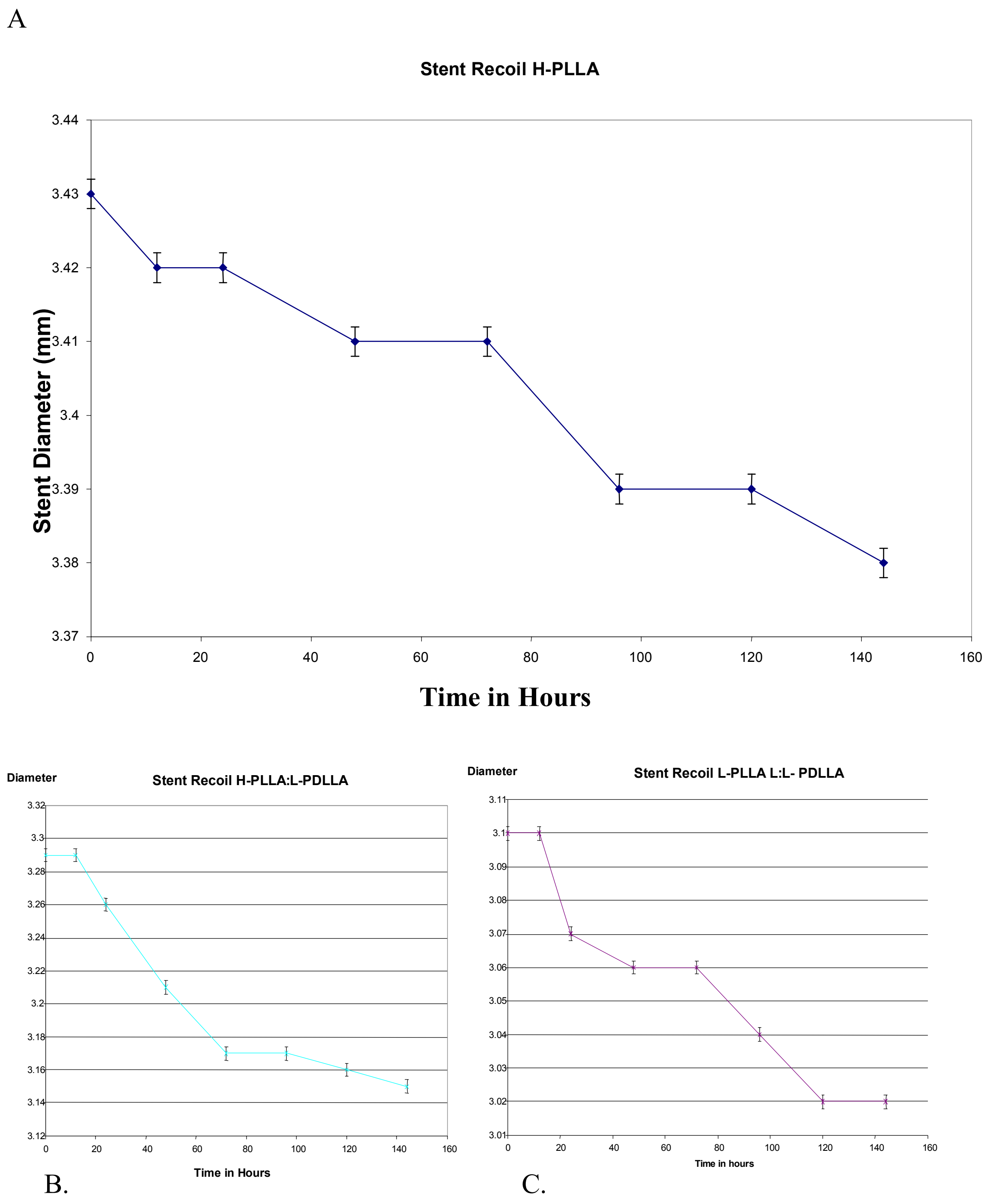


Figure 3.6 Stent recoil parameters. A. HMW-PLLA. B. HMW-PLLA: LMW-PDLLA (50:50). C. LMW-PLLA: LMW-PDLLA (50:50). n =5, mean ± SD, *p < 0.01

3.3.3 Collapse Pressures of Stents Fabricated from Blended Fibers

Stents fabricated from various polymeric blends were incubated in PBS at 37 deg C for periods ranging from 0 to 7 days. Stents made from both low molecular weight and high molecular weight PDLLA-PLLA stents lost flexibility and hardened by the end of 4 weeks incubation. It was observed that about 20 % of the stents under observation seem to have become embrittled, with a harder texture of the fiber. To verify this, 6 fibers were incubated as described for a week, and 2 out of 6 fibers had indeed become brittle. Another observation noted was the change in stent configuration; when originally deployed, they all had a circular cross section configuration. For the PDLLA based stents, the circular configuration changed towards a triangular configuration after week 3. No other cross sectional changes were observed in other samples. The third observation was the reduction in resistance to compressive pressure-induced collapse. The collapse pressure changed from about 6.7 psi to 4.9 psi at the end of 4 weeks, which amounts to a 25-25 % reduction as compared to the collapse pressures of PLLA stents that were not exposed to incubation in saline medium, as shown in figure 3.7 The HMW PDLLA-PLLA stents showed an 11% change in the collapse pressure after week one. By the end of week 2 there was a further 6% change in the collapse pressure. At the end of the study period, at four weeks, the change in collapse pressure approached 26 %, as compared to stents that were not subjected to saline incubation (controls). The stents constructed from PLLA:LMW-PDLLA blends exhibited a consistent decrease in collapse pressure: It was noted to be greater than 40 % by the end of 4 weeks' incubation. Stents made from binary PLLA blends (60:40 and 40: 60) averaged a 23-25% drop in the collapse pressures by the end of week 4.

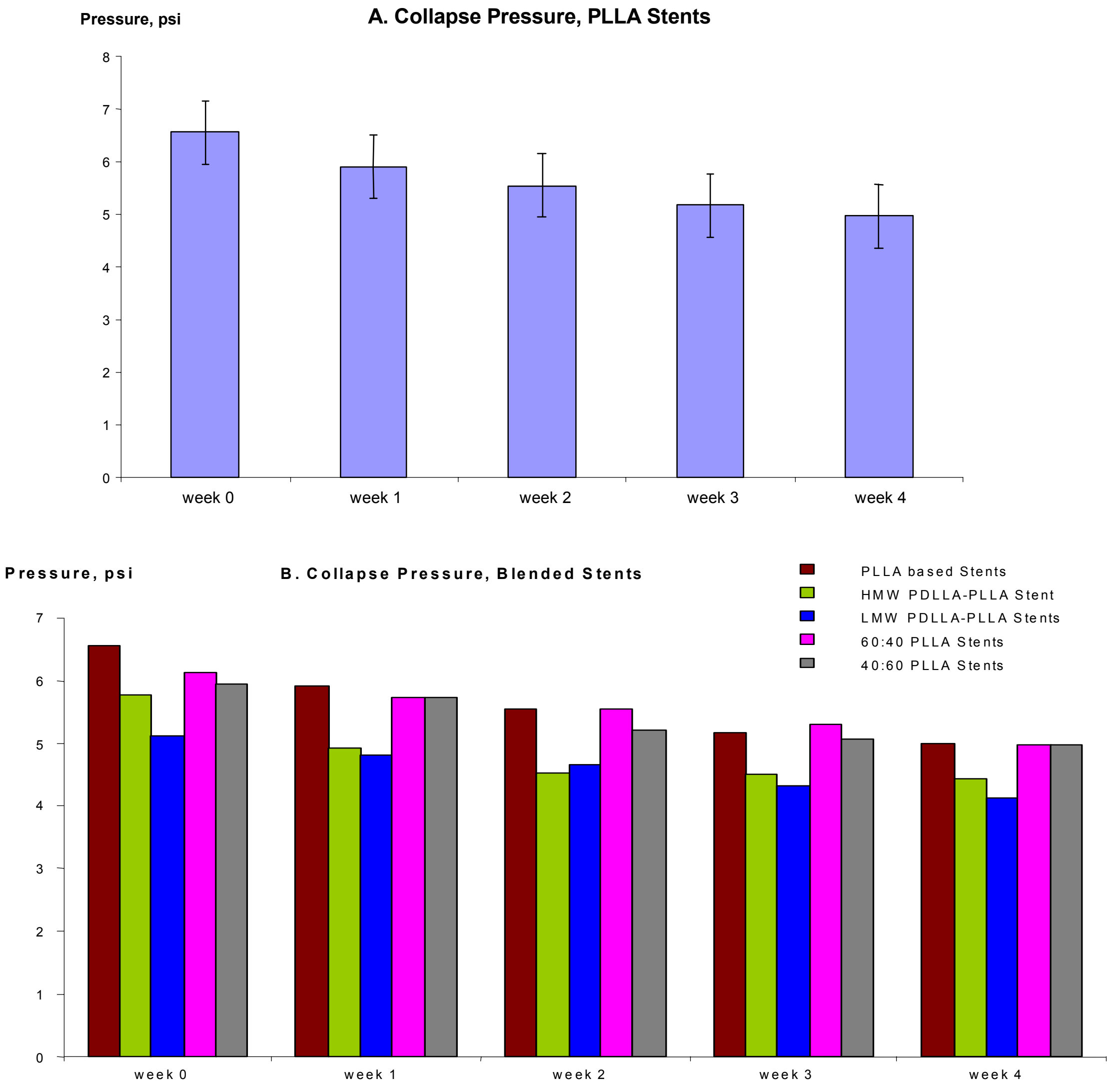


Figure 3.7 Comparison of collapse pressures of expanded stents incubated in saline. A. PLLA homopolymer. B. Blended polymers. n =5, mean ± SD.

As expected, there was a higher drop in the collapse pressures of PDLLA-PLLA based stents a compared to PLLA based stents during all portions of the 4 week period. From figure 3.9 (B) it can be seen that statistically there is not much difference in the collapse pressures of stents made from PDLLA-PLLA (50:50). The PLLA blends of (40:60) and (60:40) do indeed show a higher collapse pressure resistance than the PDLLA-PLLA blends after 4 weeks of incubation. In some cases we have seen that HMW PLLA and LMW PLLA blends with a composition of 60:40 do have a higher collapse pressure resistance than the HMW PLLA based stents. This could be a effect of fabrications process. It can be thus seen that with a increase in the LMW polymers (PLLA and PDLLA) there is a drop in the collapse pressure resistance with a increase in incubation time. From the A and B we can compare collapse pressure between blended and non-blended stents.

3.3.4 Deployment and Collapse Pressure Results for Curcumin-loaded Stents

Stents loaded with curcumin (5% by weight) needed slightly higher pressures than PLLA based stents for full expansion, all other parameters being equal. In this study, it was noted that all PLLA stents, regardless of material, blend or other configuration, achieved complete expansion at 12-13 atmospheres of pressure. When tested using the same balloon catheter, the curcumin loaded stents achieved full expansion at pressures at 12 atmospheres in one case and more than 15 atmospheres in the other. Based on my experience, garnered during two years of stent fabrication, the deployment pressures for curcumin-loaded stents are typical higher than the unloaded stents developed within allowable tolerances of radius 1.5 ± 0.2 mm and varying lengths. It is critical to note that these stents were inflated till they broke. Though from the graph it may be that curcumin loaded stents have lower inflation pressures, it is should be noted that PLLA based stents break much before curcumin. Hence it would be fair to assume that the curcumin loaded stents take a longer time to reach full inflation and hence break at higher pressures.

There is no set value to the stent length in the lab fabrication studies; it can vary between 12 to 16 mm, depending on desired characteristics. Historically variations in inflation pressures have been noted to be due to many factors. One such is glue fillet formation (that of high quality, a very strong bond will form between the longitudinal struts and coil fibers). Tension distribution within the longitudinal loop is another significant factor affecting quality. Many times I have noted the stent, after removal from the mandrel, to be more of a conical shape rather than a cylindrical shape. This could lead to inflation pressure variation for the stent.

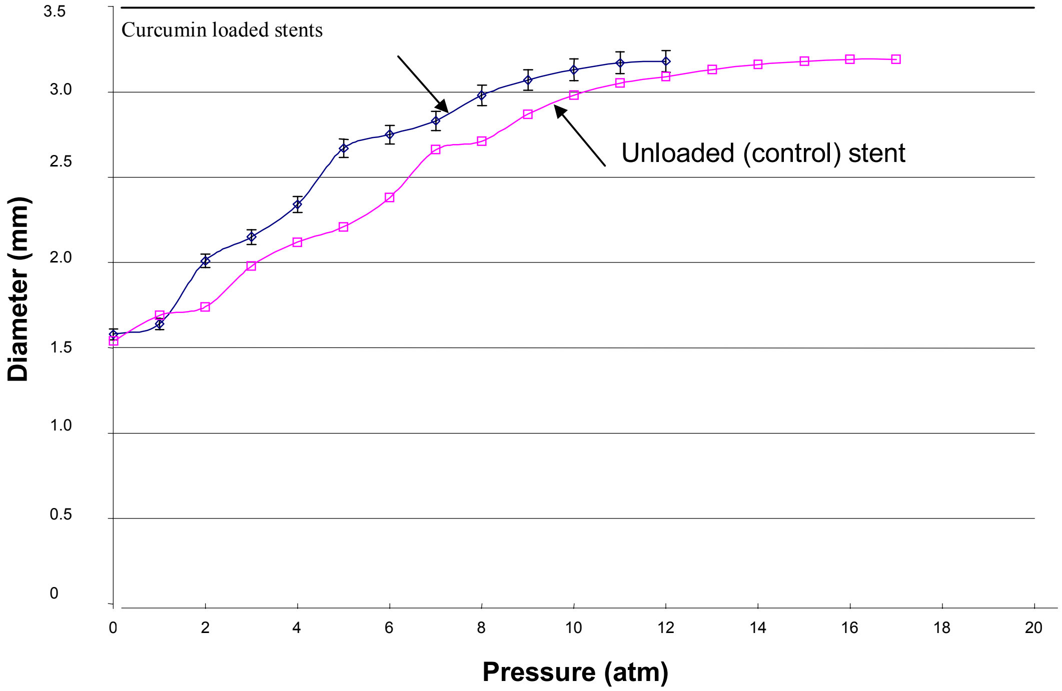


Figure 3.8 Expansion pressure profiles of curcumin-loaded stents vs (single) control. n =4, mean ± SD.

3.3.5 Collapse Pressure Measurements for Curcumin-loaded Stents:

The pressurized collapse behavior of the curcumin-loaded stents made from blends (HMW-PLLA: LMW-PDLLA) and incubated in saline is presented in Figure 3.9. This is compared with the performance of unloaded HMW-PLLA stents having similar fiber thickness, draw ratio, stent length and diameter. The rate of change of collapse pressure for the control stents appeared greater than that of the curcumin-loaded stents. By the end of week 4, there was a 20-25 % reduction for the controls, compared with a 13-15 % drop for curcumin-loaded stents.

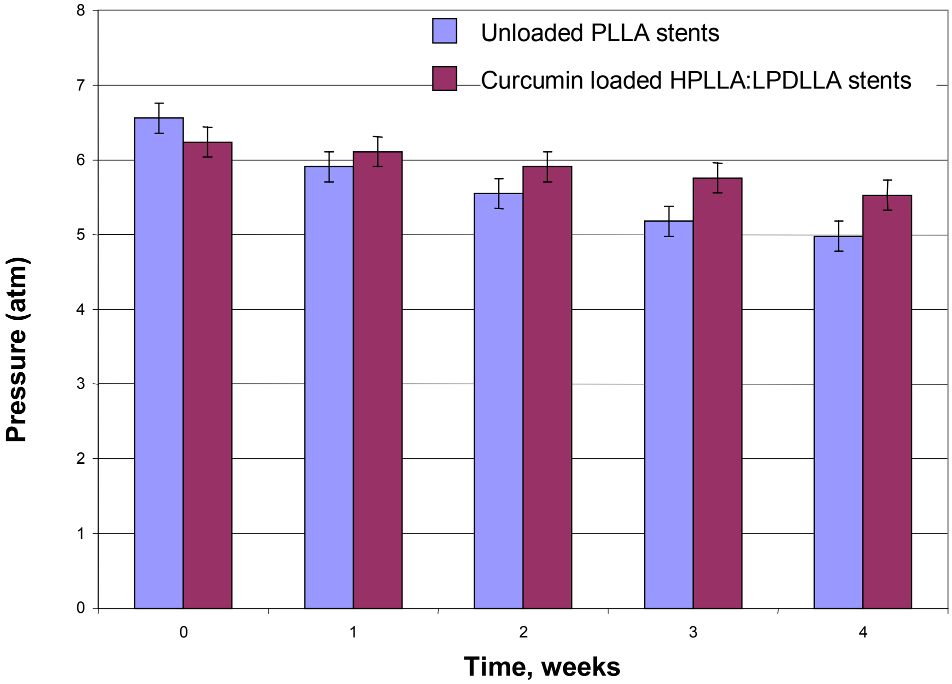


Figure 3.9 Collapse pressures of unloaded PLLA (control) and curcumin loaded HMW-PLLA: LMW-PDLLA stents. n =4, mean ± SD.

Collapse pressure studies of PLLA stents, based on different PLLA formulations, winding characteristics, gluing and axial reinforcement designs, obtained by Satasiya [2] indicate a 50% drop in collapse pressure by the end of week 13. Data from the previous report at week 4 show similar values for single ply PLLA stents to those reported in this study, with slight variations due to fabrication technique.

3.3.6 Recoil Measurements for Curcumin-loaded Stents

Immediately after removal of the curcumin-loaded HMW-PLLA:LMW- PDLLA blended stents from the balloon catheter, there was a 15% change in the stent diameter. Subsequent tests of these curcumin-loaded stents subjected to incubation in PBS for 1 week showed shrinkage by 3-5 % in diameter which was lower than the change recorded in HMW-PLLA:LMW-PDLLA blended stents without curcumin, which reduced by 6-7% in one week (Figure 3.10).

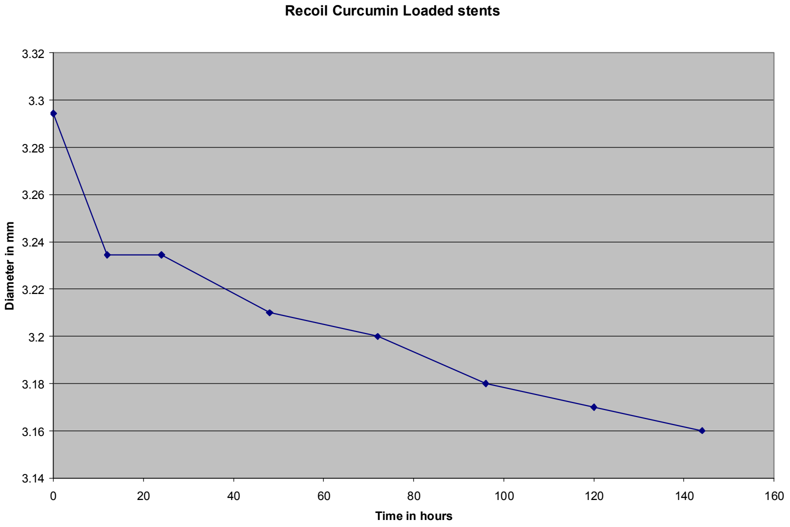


Figure 3.10 Stent recoil: Curcumin-loaded HMW-PLLA:LMW-PDLLA stents. n =4,mean ± SD <* 0.5.

Comparing results with those of Section 3.3.2 we can see that HMW-PLLA stents, incubated under similar conditions without curcumin, had average recoil of about 3-4 % in a week (Figure 3.11). Hence it can be said that presence of curcumin reduces the elastic recoil in these stents.

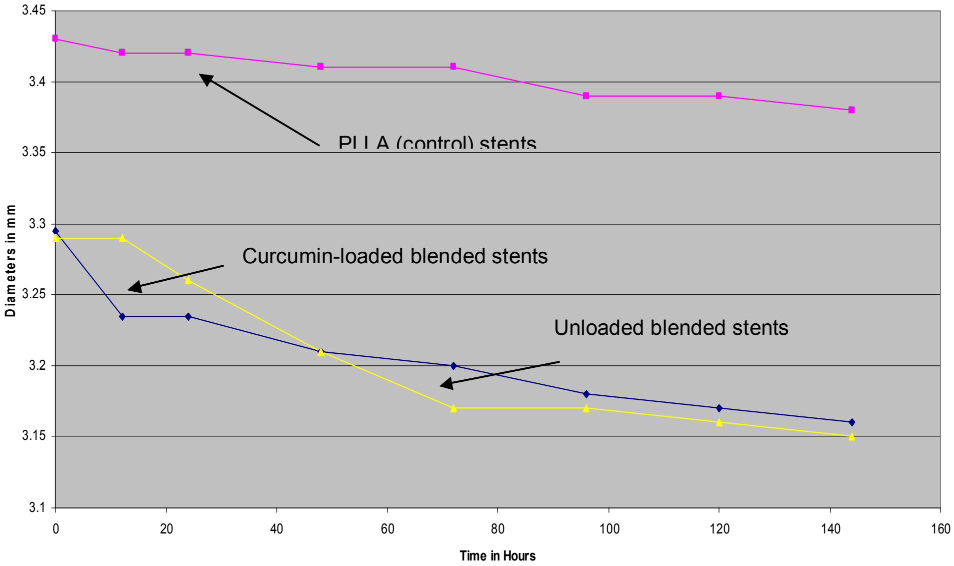


Fig 3.11 Stent elastic recoil comparison.

3.4 Thermal Analysis of Stent Fibers

Differential Scanning Calorimetry was used to determine physical properties of the blended polymers, melting point (Tm), glass transition temperature (Tg) and % crystallinity.

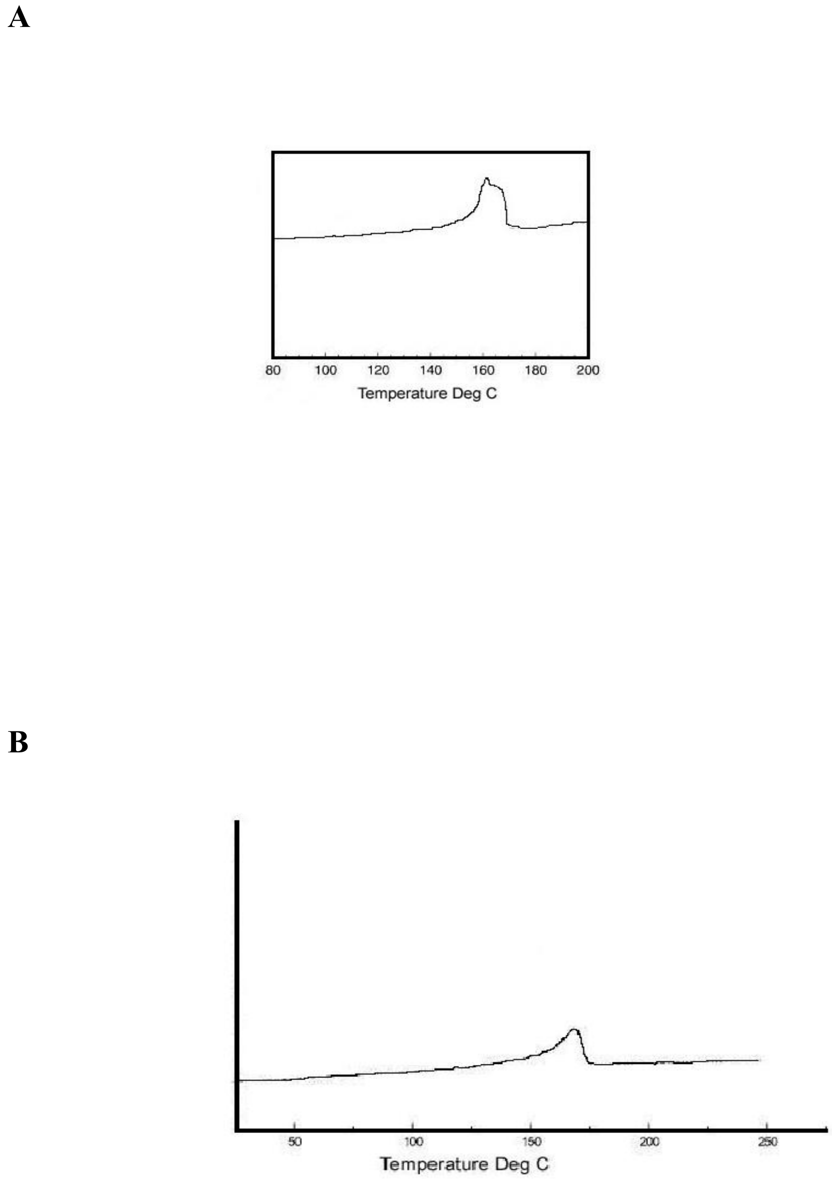


Figure 3.12: DSC Thermograms of blended fibers. A. HMW-PLLA: LMW-PDLLA 50:50 blend, amorphous. B. LMW-PLLA: LMW-PDLLA 50:50 blend, amorphous.

The DSC analysis was used to determine the thermal characteristics of the HMW-PLLA:LMW-PDLLA and LMW-PLLA:LMW-PDLLA blends with mixing ratios of 50:50. The Y-axis is the heat flow and the X-axis is the temperature in deg C. Please note that the temperature scales were modified by standard means in order to get the pronounced effect of the valleys and peaks in both thermograms. A single glass transition temperature was observed in both cases, indicating that the amorphous portions of the two polymers were miscible with each other. It was observed that with the increase in the LMW-PLLA component there were downward shifts in both the blend glass transition temperature and melting point (Fig. 3.12 and Table 3.3)

Table 3.3 DSC parameters for extruded fibers consisting of HMW- & LMW-PLLA 50:50 blends with LMW-PDLLA.

50:50 Blend	Glass transition, Tg (°C)	Melting, Tm (°C)
HMW-PLLA:LMW-PDLLA	52.27	172.35
LMW-PLLA:LMW-PDLLA	43.51	169.12

In other words, as expected, increasing HMW-PLLA content increased both Tg and Tm. The crystallinity of the fibers (drawn 4:1) was also determined to see how the lower molecular weight polymer affects the crystallinity of the blended fiber. The degree of crystallinity was determined from the respective enthalpy of crystallization (ΔH_c) and the enthalpy of fusion (ΔH_f) using the relationship:

$$\% \text{ Crystallinity} = \frac{(\Delta H_f - \Delta H_c)}{\Delta H_f^{'}} \times 100$$

where $\Delta H_f^{'}$=93.7 J/g is the enthapy of fusion of 100 % crystalline sample of PLLA [54].

Using these relations and the DSC data it was determined that the % crystallinity for the higher MW PLLA-containing polymer blend is 52.13% as compared to the lower MW blend, which is 47.72%. Additives and/or contaminants can lead to formation of erroneous readings present within the thermograms. Cold crystallization endotherms are observed before the melting peak for PDLLA-PLLA blends is reached.

3.5 Mechanical Analysis of Stent Fibers

We measured the uniaxial stress vs. strain properties of fibers made from binary PLLA: PDLLA blends (HMW-PLLA:LMW-PDLLA and LMW-PLLA:LWM- PDLLA). Young's modulus, ultimate tensile strength and % strain at break are reported. Based on the anticipated availability of DSC data for a larger number of samples, blends were prepared and melt- extruded to obtain amorphous fibers. Table 3.4 displays the results. It can be seen that as expected, ultimate tensile strength was highest for HMW-PLLA homopolymer. There were significant differences in the tensile modulus, strength and strain at break for the two fiber blends.

Table 3.4 Mechanical properties of PLLA-PDLLA blended fibers. n =4, mean ± SD.
* $p<0.01$ wrt H-PLLA blend and H-PLLA control.

Blend	Young's Modulus (MPa)	Tensile Strength (MPa)	Strain (%)
50:50 (H-PLLA:L-PDLLA)	7,954 ± 912	181 ± 27	8.1 ± 1.6
50:50 (L-PLLA:L-PDLLA)	4,853 ± 861*	139 ± 21*	4.5 ± 2.3*
100:0 (H-PLLA)	8,012 ± 856	192 ± 25	9.7 ± 1.7

3.6 Blend Degradation Tests

Mass loss is an important criterion that characterizes the extent of polymer degradation in a different and more revealing way than molecular weight change. The

50:50 blend of HMW-PLLA:LMW-PDLLA, subjected to incubation in a 37 deg C saline solution for a period of 4 weeks, showed a change in weight loss of approximately 12 % (Fig. 3.13 A). As expected, the incubated LMW-PLLA:LMW-PDLLA 50:50 blend showed a larger change in weight at the end of 4 weeks, 20-25% (Fig. 3.10 B). The weight loss noticed in HMW-PLLA fibers is about 4% (Fig. 3.10 C). The incubated HMW-PLLA: LMW-PLLA (40:60) and HMW-PLLA: LMW-PLLA (60:40) blended fibers showed similar weight loss, 12-14%, at the end of 4 weeks.

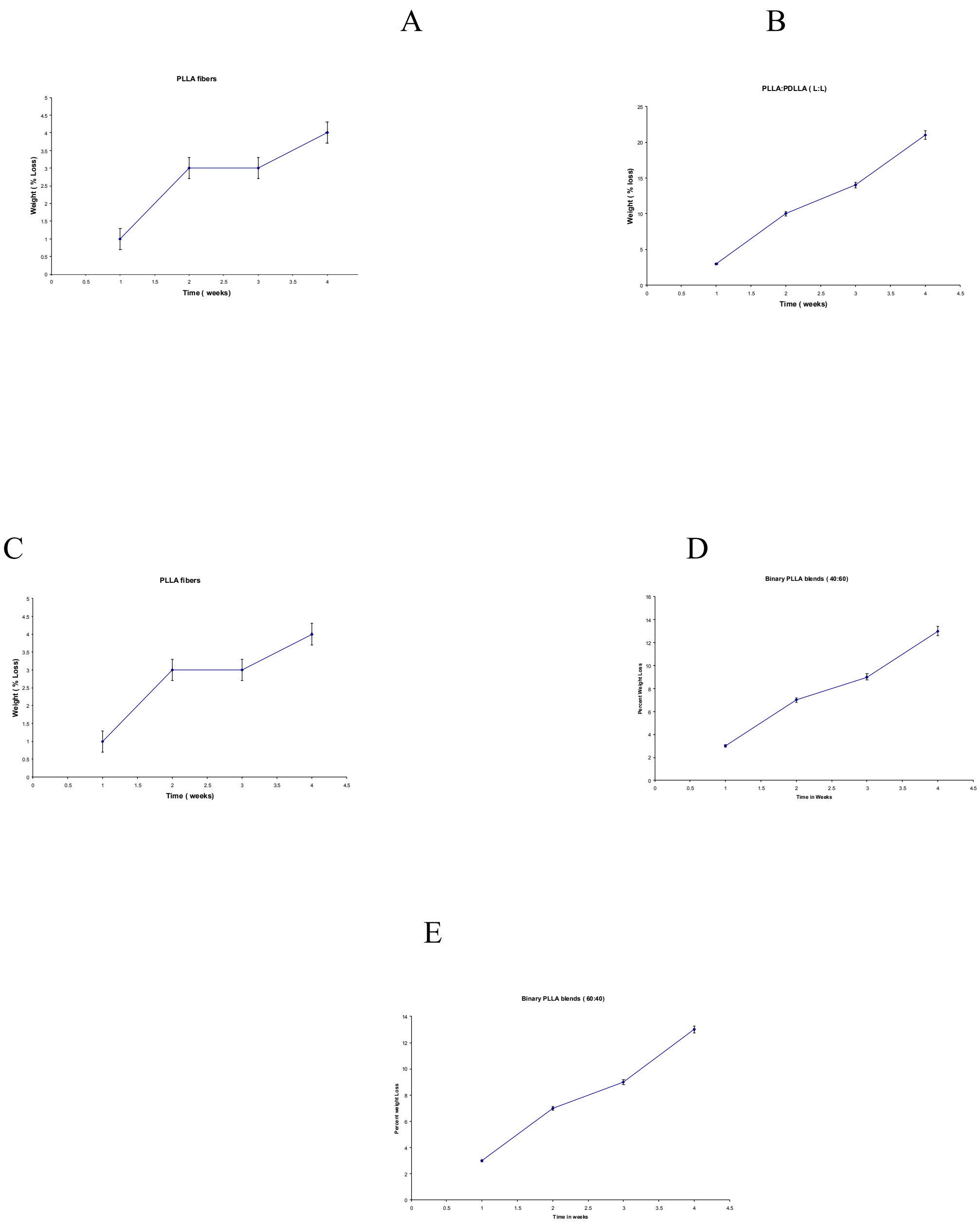


Figure 3.13 Blend Degradation plots n=10 p<*5 Mean ± SD
A H-PLLA:L-PDLLA (50:50) B. L-PLLA:L-PDLLA (50:50) C. H-PLLA D. H-PLLA:L-PLLA (40:60)E. H-PLLA:L-PLLA (60:40)

It was also noticed that the LMW-PLLA and LMW-PDLLA fibers became very brittle. They broke consistently as they were removed from the PBS solution at the end of 1 week, even using tweezers. As compared to the PLLA based fibers the PDLLA lost an average of 15% extra weight. All fibers were similar in weight with relatively small deviations in the base weight. In order to test the feasibility of the blended fibers in fabricating stents, we prepared 10 stents and subjected them to PBS at 37 deg C incubation for one week. The stents were of a circular configuration before incubation. After one week they were dried at 37 deg C for a day. It was observed that the stent had lost its circular shape and had conformed to a triangular configuration. However further analysis regarding the mechanical strength and collapse pressure of this type of stent needs to be done.

CHAPTER 4

DISCUSSION

Stent implantation following PTCA is a common procedure performed in hospitals throughout the world. Many clinical studies of stent efficacy and safety have been done in the last fifteen years. These studies are increasingly focused on long-term results for drug-eluting metal stents, covering a wider range of indications, including smaller coronary vessels, treatment of vessel restenosis, etc. Various combinations of stent designs and stent delivery components, and different drug eluants, have undergone clinical studies in these regards in the past 5 years, and more detailed studies are planned or underway. Despite the focus on longer term results with current stent platforms, common problems remain with these stent designs. First and foremost, these are permanent platforms, that may and probably do outlive their usefulness. Additional problems include lack of flexibility, limited expandability and low compression resistance. Su [1] and based on the Su design, Satasiya [2] and Gampa [3] have developed and improved a novel coil design for biodegradable polymeric coronary artery stents which give to this polymeric stent design category important advantages: better flexibility and lower pressure required for expansion whilst maintaining an acceptable collapse pressure resistance. These features are expected to be of increasing importance as clinicians address the treatment of vulnerable plaque, thought by many to be a primary cause of myocardial infarction in the setting of advanced coronary arterial disease. In this regard the development of a temporary stent requiring low expansion pressure and with capability for the localized delivery of therapeutic agents over a reasonable period of time may stabilize vulnerable plaque and reduce the potential for rupture, thrombosis, etc.

This thesis attempts to build on the low pressure deployment characteristics of biodegradable single ply stents as developed in [1] and [2] by providing better gluing, degradation rates, and improved reservoir capacity for drug loading, as well as using techniques to enhance the wall apposition characteristics of the stent.

4.1 Modification of the Gluing Technology:

The PLLA glue designed and developed by Su [1] for "welding" the reinforcement fibers together to the stent body proved to have significant defects during many deployment and collapse pressure tests performed early in this thesis research. It was observed that many, if not most of the stents underwent failure at some weld points. While not all stent weld points failed, only one failure would be cause for stent design rejection in animal and clinical trials, since the failure could lead to incomplete apposition, thrombosis and restenosis. Tests also revealed that while the glue appeared to be applied uniformly, and even though the welds often did not break at the attachment points, the quantity of glue applied was insufficient to form a strong bond between the peripheral loops and the reinforcement fibers. After repeated observation it was established that the weld point was unable to withstand the stresses developed during the stent expansion phase. One method to deal with this problem is to increase the quantity of the glue in the fillet at the stent fiber crossover points. This was tried, but had undesired consequences, since almost immediately one would notice a partial "dissolving" of the fibers, since the larger applied volume of glue included a higher quantity of chloroform solvent. It was also revealed that the chloroform solvent did not evaporate completely in a suitable period of time. A slow erosive action could be seen, in which the solvent actually “ate“ away some of the fibers, weakening the stent and resulting in non-uniform expansion and/or failures in this or subsequent collapse pressure experiments. A large percentage of these “reinforced weld” stents failed during the deployment stage, never reaching the stage of testing for collapse pressure.

Thus new bioresorbable polymer glues were designed, using the combination of low molecular weight and high molecular weight PDLLA and PLLA. The main advantage observed in these studies was a 50% reduction in the amount of chloroform needed to develop the glue, as compared to the quantity needed to prepare the former PLLA-based glue. Subsequent testing also revealed that the glue had better wetting characteristics to the PLLA fibers, good adhesive characteristics and minimal “threads” formation. It was also revealed that a high quantity of glue at the fiber crossover point did minimal damage to the fibers, although some cases proved otherwise. Those failures can be attributed to factors such as miscalculation of chloroform content, thinner fibers, and fiber winding issues, which lead to mismatch of the top and bottom diameters of the stent. While these features are advantageous for long-term stent performance, the modified glue definitely wasn’t as strong as the original glue prepared using HMW PLLA. A stronger glue is advantageous during deployment, the time of maximum stressing of the fibers [74]. Nevertheless, it appears that improved fillet formation, and other design improvements, e.g., two ply designs, may permit the modified glues to withstand the intensive stresses exerted at the cross-over points.

4.2 Heating during Stent Deployment

The single ply stent designed by Su and improved by Satasiya reverted to a triangular cross-section after deployment rather than maintaining a circular one [1,2]. This was avoided in the stronger two ply stent design [54]. As the single ply inner coil stent is expanded by balloon pressurization, the three inner (peripheral) coils are converted to a single coil. A 360 degree twist occurs in each inner coil in the last stages of coil contraction before it finally merges into the main coil at a point halfway between the longitudinal reinforcing fiber cross-over points (placed 120 degrees apart). As explained by Welch [67], the torsional stresses imposed on each coil at this juncture exceed the torsional yield point, resulting in plastic deformation of the outer portion of the fiber, thus incorporating a permanent twist. This “torsional preloading” beneficially increases the collapse pressure resistance, but perhaps at the expense of a noncircular cross section caused by kinking. Even though only the outer portion of the fiber cross section undergoes plastic yielding, we can conclude reasonably accurately that this is a site for stress accumulation as degradation proceeds. Even though the kniking and formation of a triangular cross section may improve vessel grip, thus preventing flow-induced stent slippage, the triangular cross section prevents the desired perfect apposition to vessel walls. This may cause eddies near the gaps between the stent fiber and the wall, promoting rapid thrombus formation. Furthermore, loss of stent apposition has recently been observed clinically, by IVUS, as a late recoil phenomenon [10]. Further unequal stress development in the fibers of the triangular cross section stent might cause the stent to fail at less than nominal radial inward pressures as exerted by the vessel wall.

Thus, mechanical and thermal annealing techniques were studied in order to develop means to achieve a circular cross section upon balloon expansion. Balloon expansion was conducted with the expansion fluid (in this case water in the balloon catheter) heated to the vicinity of the polymer glass transition temperature. This was done in order to initiate reorientation of the polymer molecular structure, induced mechanically by balloon pressurization. Reorientation of the polymer chains near Tg would relieve the stresses built during stent deployment. When the balloon is deflated and the stent is taken off the catheter, the relaxation of the built-up stresses will prevent the stent from re-organizing towards a triangular cross section. As seen in the results section, stent kinking caused by permanent plastic torsional deformation was progressively reduced as balloon water temperatures rose above 37 deg C, finally disappearing for temperatures above 50 deg C.

However this heating maneuver also has disadvantages, the main one being increased temperatures for the vessel wall and the blood. The Igaki/Tamai stent, however used temperatures in excess of 60 deg C for stent deployment. Their studies do not show damage to the vasculature following deployment at those temperatures. The stent heating technique may not be favorable for two-ply PLLA stents, such as those developed by Gampa [54]. The stresses developed at the bonds in that instance can be very high. At this point in time we do not have an accurate idea of the stress build up, due both to to lack of a FEA model for the two ply stent design, and systematic research into the heating effects in that case. The finite element modeling may reveal useful insights relating to stress distribution and torsional preloading in that case, as it has already done for the single ply stent case.

4.3 Thermal Characterization of Blended Polymers

Su successfully loaded LMW PLLA fibers with an anti-proliferative drug, curcumin [1]. Gampa tried loading curcumin into HMW-PLLA:PEO-PLLA blends, but was unsuccessful (personal communication). Modification of the crystalline structure of PLLA, such that the melting temperature is reduced to enable loading of curcumin below its denaturation temperature was the motivation behind that effort. Using blends of PEO and PLLA, crystalline structure modification can be achieved, but it compromises the mechanical strength of the polymer, making it unsuitable for vascular applications. Furthermore, PEO is immiscible when added to the commercially available PLLA polymers. PEO can be blended more efficiently when the PLLA polymer is synthesized in-house [75]. The polymers obtained for this research are purchased without much knowledge of the synthesis techniques, additives and relevant information pertaining to the doctoring of the polymer for specific needs. Small experiments performed over the duration of this research did lead us to believe that HMW or LMW PEO added with the conventional techniques mentioned in section 2.3 are not suitable. The polymers were deemed quite immiscible. One way to deal with this issue was by tweaking certain conditions, including the addition of surfactants to enhance miscibility.

Blending of low and high molecular weight PLLA with PDLLA is another potential method to increase drug reservoir capacity. PLLA is a semi-crystalline homopolymer. PDLLA exhibits both random distributions of the isomeric forms of lactic acid. Hence PDLLA is unable to form organized crystalline forms. Tight packing of the PLLA and PDLLA polymer chains within the matrix can be one of the reasons as to why we fail to observe any phase separation due to absence of cohesive or repulsive forces present between the polymer chains [72].

We can see from the DSC thermograms of section 3.4 that the 50:50 HMW-PLLA:LMW-PDLLA and LMW-PLLA:LMW-PDLLA blends exhibited different glass transition temperatures. The higher quantity of the low molecular weight polymers (PLLA and PDLLA) in the latter case tends to decrease Tg. When one considers in addition the amorphous structure of PDLLA it is seen that crystallinity is decreased, and thus, Tg decreases as well. The LMW-PLLA:LMW-PDLLA blend also exhibited a cold crystallization exotherm beyond Tg and an asymmetric melting peak. The recrystallization process observed when the HMW-PLLA:LMW-PDLLA blends were heated beyond Tg might have resulted in the formation of smaller crystallites that were not incorporated into the larger crystallites of HMW-PLLA. This produces a lower Tm than for HMW-PLLA, which is rich in crystallites [62]. The asymmetry in the melting peak might be because of the difference in the melting temperatures of the two different crystal sizes present in the blend. Other reasons for the differences in Tg (and Tm) may be due to additives. Literature reports [60,61] mention that PLLA and PDLLA do not blend without the use of surfactants. These and other additives blended in during synthesis could improve the miscibility of the polymers as reported in [76]. However we do not know what effect these surfactants might have on this, and other drug loading parameters (safety, efficacy).

As we have used PLLA and PDLLA to prepare the all the blends, there are minimal repulsive/cohesive force interactions among the polymer chains, and hence we do not see phase separation. It is fair to assume that PDLLA and PLLA indeed are miscible, as suggested by the DSC thermograms. If they were immiscible then the amorphous phase would have maintained the respective properties of both polymers, and we would have seen two separate glass transition temperatures in the same thermogram.

First consider a relatively simple case of blends of the same polymer, with only MW variation. From Table 4.1, data extracted from the Fig. 4.1 DSC thermograms obtained by Gampa [54] for various blends of HMW and LMW PLLA, (all materials supplied by the same producer), we reason that higher molecular weight components better support crystallite formation and hence, higher melt temperatures.

Table 4.1Thermal characterization of HMW: LMW PLLA blends (from Gampa [54])

H-PLLA:L-PLLA	Glass transition, Tg (°C)	Melting, Tm (°C)
40/60	53.13	171.88
60/40	53.52	178.88

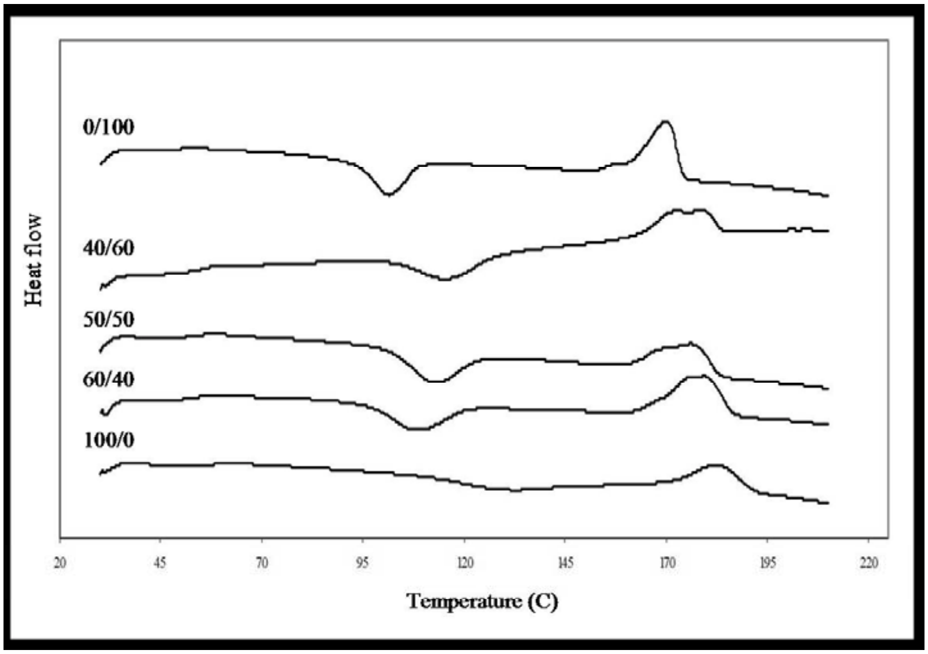


Figure 4.1 Thermograms for various HMW: LMW PLLA blends (from Gampa [54])

Now consider the more complex case of HMW and LMW blends of two different polymers, PLLA and PDLLA. Blends prepared using different percentages of polymers have been shown to be effective in high-mechanical load orthopedic implants [3,63]. Thus blends might improve mechanical properties whilst allowing higher reservoir capacity for drugs such as curcumin. Table 4.2 presents taken for PLLA:PDLLA sample blends, created at the same time as those presented in section 3.4, but with DSC analysis at different times at two different labs. The first two rows represent blended fibers that were analyzed immediately after melt extrusion and drawing. The next two are for fibers stored for 3 months prior to DSC analysis. The last two row entries are for fibers from the same extrusion run, but stored 3-4 months prior to analysis at Dr. D’Souza’s lab at UNT, with a different instrument and operator. The thermograms corresponding to these last two entries are given in Fig. 4.2. The UTD HMW-PLLA:LMW-PDLLA fresh fiber data show an identical Tg as given in Table 3.3, but a substantially higher lower Tm. The fresh fiber Tg for LMW-PLLA:LMW-PDLLA is substantially higher, and the Tm substantially lower than the corresponding values in Table 3.3. The 3 months’ storage data (rows 3 & 4 of Table 4.2) show similar Tg values to the fresh fiber, but higher Tm, close to the corresponding values in Table 3.3. The data from the UNT analysis indicate lower Tg values than for any corresponding UTD analysis, but similar Tm values. How to explain these results?

Table 4.2 Thermal characterization of fresh and aged HMW & LWM PLLA: PDLLA (50:50) blend fibers. Data from DSC analyses run in two different labs. UTD: University of Texas at Dallas; UNT: University of North Texas.

Blend Type (50:50)	Glass transition, Tg (^{o}C)	Melting, Tm (^{o}C)
H-PLLA:L-PDLLA (fresh, UTD)	52.27	168.35
L-PLLA:L-PDLLA (fresh, UTD)	49.51	159.35
H-PLLA:L-PDLLA (3 mos, UTD)	52.20	173.32
L-PLLA:L-PDLLA (3 mos, UTD)	48.23	169.2
H-PLLA:L-PDLLA (3+ mos,UNT)	49.4	172.1
L-PLLA:L-PDLLA (3+ mos,UNT)	47.2	169.5

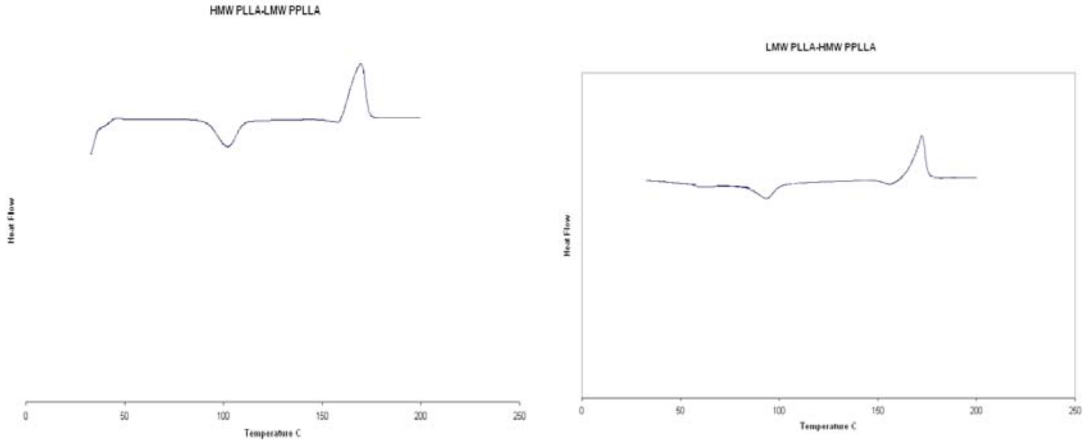


Figure 4.2 DSC thermograms of PLLA: PDLLA blends analyzed at UNT after 3-4 months' storage.

Other than operational differences (unlikely to yield changes, if contamination by improper handling is avoided), many polymer structural factors could lead to the observed change in the melting point. The incursion of moisture could have affected the amorphous region of the blend, particularly so for the LMW-PDLLA. This would lower MW, and would also reduce crystallinity, hence reducing Tg. This was observed as indicated in Table 4.2. At the same time, due to the increase of polymer chain mobility beyond Tg, there might have been a realignment, with time, of some amorphous segments into crystalline structures [62, 65]. In theory, the blends could have developed increased numbers of chain ends that were included in crystallites, thereby altering the surface free energy, affecting an increase in Tg and Tm [55]. Furthermore, the higher Tm observed for HMW-PLLA: LMW-PDLLA (Table 4.2): could also be due to formation of larger crystallites because of the larger polymer chains. These could require higher temperatures to uncoil and mobilize. Thus there are reasons to support some, but not all of the observed results. An increase in Tm consistent with this excplanation was observed. But as noted, there was a contrasting, otherwise unexplained reduction in Tg.

Many factors in polymer stereocomplex formation are dependent on the molecular weights and processing parameters of PLLA and PDLLA. Von Recum et al [65] reported differences in Tm due to the presence of different crystallite sizes for different polymers, including PLLA. During polymerization of poly-lactates it was observed by Tsuji that different types of crystallites may be formed, which can be homo-crystallites [62]. When PLA L and D chains are of high molecular weight, then homo- as well as stereocomplex crystallites are formed. These are affected by various parameters like molecular weight, (optical) purity and the mixing ratios of isomeric chains [63]. Re-crystallization can be affected by the presence of these different crystallites.

From the results of section 3.4 it is seen that the addition of the amorphous, low molecular weight PDLLA directly reduces Tg and Tm. Similarly the lower molecular weight PLLA will also increase the amorphous content, further reducing crystallinity, thus reducing Tg and Tm. Smaller chains more readily align along the axis of a polymer fiber when heated above Tg. Higher percentage crystallinity could result from the presence of larger numbers of smaller, but more organized polymer chains. But as noted, the Tg values of Tables 3.3 and 4.2 are not all consistent with this surmise. It may be that the unfavorable environmental conditions, e.g. humidity, temperature and contamination could have a significant effect on the amorphous regions of the blended fibers. This may have lead to degradation. This could help to explain the increase in the melt temperatures of the blends after a period of storage, as these partially degraded polymers may have contained higher percentages of crystalline polymer. Clearly, further research is required.

4.4 Mechanical Characterization of Blended Polymer Fibers

The mechanical properties of a polymer such as tensile strength, Young's modulus, and tensile strain at break depend on the intrinsic polymer structure. These properties undergo changes if the fiber undergoes degradation. The high molecular weight of PLLA in a blend might be responsible for its high tensile strength (181 ± 27) and low strain at break (8.1 ± 1.6) compared to the fibers of LMW-PLLA:LMW-PDLLA blends that are 139 ± 21 and 4.5 ± 2.3, respectively. Under constant strain all the fibers made from blends exhibited ductile failure, which is the preferred mode of failure for fibers used for stent fabrication. PDLLA does not contain a crystalline structure and is highly amorphous. This increased amorphous portion in the blend due to the presence PDLLA may play a role to increase the ductility and lower tensile strength and % strain. It can be said that as the content of HMW PLLA increases the crystalline content increases which increases the toughness of fibers as can be seen with a higher elastic modulus.

4.4 Mechanical Characterization of Fabricated Stents

4.4.1 Stent Deployment Pressure

At this point in time it is unclear if high deployment pressure will benefit or hinder the clinical outcome [64-66]. Metallic stents are deployed at pressures lower than 5 atmospheres in order to minimize the wall stress and reduce subacute thrombosis. Conflicting results are published where, in some cases, angiographic studies in humans led to the conclusion that high inflation pressure could result to a 30-60 % lumen loss as compared to low inflation pressures [67]. In a prospective study, Dirschinger et al found no difference in clinical event rate, minimal lumen diameter or restenosis rate between the high and low pressure implantation techniques [68]. Most clinicians prefer low inflation pressures. Many also recognize the possibility of thrombus induction due to stent recoil may be associated with lower inflation pressure.

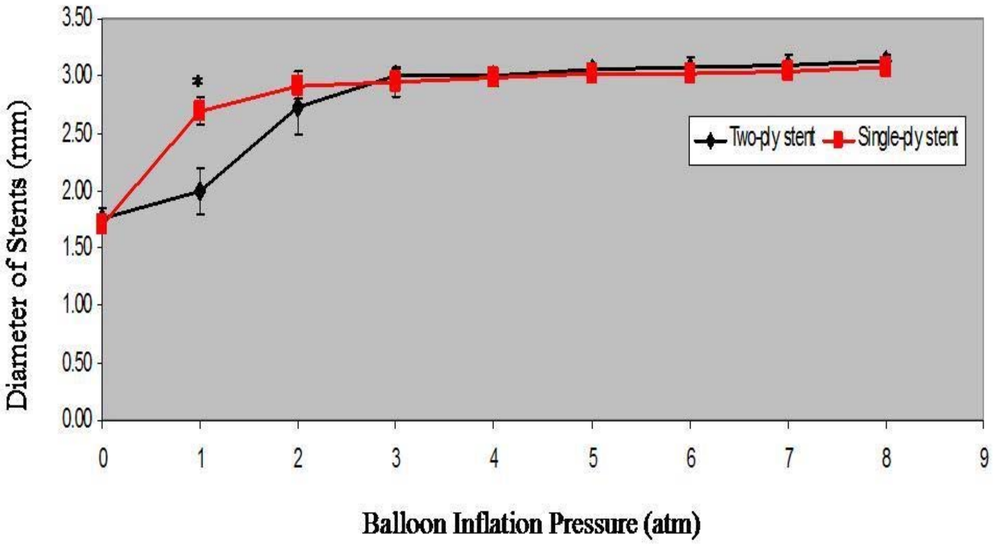


Figure 4.3 Changes in stent diameter with increasing balloon inflation pressure, n=6, mean ± SD, $*p < 0.05$. Reproduced with permission [54].

From the studies done in the recent past and our own lab experience [1, 2, 54] it can be confirmed that the polymeric stents provide a margin of flexibility in deployment pressure. From Figure 4.2, incorporated from the Gampa thesis [54], single ply PLLA fiber stent inflation was achieved between 2.5 and 3 atmospheres. With the fabrication methods employed in that and previous work, it is probably difficult to obtain consistent inflation pressures. Many reasons for such inconsistency can be offered, including fabrication method tendencies of individuals, fiber thickness and crystallinity, glue quantity at the struts, etc. The stents described in this research effort are made from the blended polymers (HMW-PLLA and LMW-PDLLA) achieved full deployment at 3.7 to 4 atm. Stents made from H-PLLA require 4 to 4.5 atm to reach 100 % expansion in this series. About 90% of full expansion is reached between 2 to 2.4 atm in both cases. The lowest inflation pressure was 2.5 atm for L-PLLA:L-PDLLA blends. These results suggest that a relatively low molecular weight PDLLA component within the polymer matrix improves fiber flexibility as compared to H-PLLA. This may provide a chance to minimize the injury sustained by a vessel due to higher pressure needed for the inflation of the stent. The many noted factors influencing inflation pressure argue for a stent production protocol capable of defining tolerances for glue composition, weld fillet size and position, fiber tension distribution during winding, etc. It is recommended to not claim inflation pressures for less controlled fabrications with confidence. This feature is critically important when the stent is being considered for implantation in vessels with vulnerable plaque. Thus low-pressure blended polymer stent design may be placed with less danger of rupture of vulnerable plaque lesions. However intensive testing, experimentation and analysis need to be done to confirm the above.

4.4.2 Stent Collapse Pressure

There is no set limit for the collapse pressure parameter which a vascular stent must withstand [1]. The accepted notion is that the higher the collapse pressure, the better. For the inner coil stent design investigated in our lab, the collapse pressure can be increased by employing higher fiber plies [1,2,3,61]. However the complexity of the design increases with the increase in ply; other critical factors may undergo significant changes, including lateral spreading of the fibers post deployment, loss of apposition and inflation pressure. In lieu of increasing fiber ply, one may increase fiber diameter; however this compromises the flexibility and increases the stent inflation pressure. Satasiya [2] states that torsional pre-loading can, in effect, be incorporated during fabrication and hence the stent can withstand higher collapse pressures. Gampa et al [54] in her two-ply stent design points to an increase in collapse pressure. However in the research of this thesis, the collapse pressure of HMW-PLLA:LMW-PDLLA blended single ply stents was 3 to 5 % lower than the collapse pressure of HMW-PLLA single ply stents. It can be speculated that the collapse pressure can be increased with an increase in HMW-PLLA fiber content. It is difficult to say how much of this can be attributed to the torsional preloading since many small manufacturing errors such as varying fiber thickness, glue inconsistency are some of the other issues that can change the collapse pressure values.

A study done by Rieu et al [69] stated a numeric value of 4.35 psi (0.3 atm) that may be considered the minimum compression resistance required for an intracoronary stent. The stents developed in this thesis easily surpassed this value. After incubation for 1 month at 37 deg C the stents maintained a good collapse pressure resistance of about 4 psi, which is in compliance with the FDA guidelines for stent compressive forces. However it can be speculated based on detailed observations of stent shape, degradation rate and temperature that the stents may lose a large percentage of the scaffolding function (e.g., collapse resistance) after 2 months incubation.

4.4.3 Curcumin Loaded Stents: Collapse, Deployment and Recoil Pressures

As observed by Su [1] the mechanical properties of PLLA stents improved with curcumin addition. That work was done with low MW PLLA. But recent reports on stents fabricated with high MW PLLA suggest that curcumin impregnation also enhances the mechanical characteristics [2]. PLLA is brittle in the amorphous state. PLLA brittleness was reduced by a relatively large margin due to impregnation of curcumin, suggesting the additive served as a lubricant. The mechanical strength of PLLA also depends on its degradation characteristics. Independent researches have lead to the observation that curcumin retards the rate of degradation. The commonly accepted degradation mechanism for PLLA is cleavage of the ester groups. As degradation of hydrophobic PLLA proceeds, the hydrophobic curcumin is released. But the hydrophobicity of both polymer and drug may lead to the formation of a curcumin coating around the PLLA fiber. Curcumin has been documented to have free radical scavenging ability. This scavenging may remove OH groups from the polymer surface and otherwise limit bound water, thus protecting the polymer esters and thereby slowing down hydrolysis. This can be one of the possible reasons for retention of high mechanical strength. This extends over to higher stent recoil resistance and collapse pressure resistance than stents designed from other polymer blends. Testing stents with curcumin loaded within has not been performed before. However it would be only fair to reason that slow degradation may not be clinically effective since the maximum utilization of the implant is within the 1st 3 months. Hence PLLA has been shown to have a degradation age of about 2 years by many independent researchers. PDLLA-PLLA blends as discussed earlier have shown faster degradation properties. Significant loss of mechanical strength is also to be expected.

Incorporation of curcumin with the PDLLA-PLLA blend might provide with the ideal balance of a faster degrading polymer blend with ideal therapeutic qualities and sufficient mechanical strength to withstand pressures exerted by the vessel wall until geometric remodeling of the vessel wall.

4.4.4 Stent Recoil

When a stent is placed inside a stenotic artery it is expanded to achieve a diameter close to the desired final lumen diameter of the vessel, but however it can certainly undergo a reduction in the diameter in some cases this is up to 13% of the original diameter post deployment. Many factors determine the recoil parameters. In this study there is a change in the stent diameter by 2 to 3 % in HWL-PLLA based stents and by about 6 -7 % in HMW-PLLA:LMW-PDLLA blended stents. However the significant reduction in recoil in the later phases of the saline incubation of the HMW-PLLA:LMW-PDLLA blended stent (Fig.3.6B) can be attributed to the decreased degradation rate, owing to the higher percent crystallinity of HMW-PLLA. Increasing the LMW-PLLA content in the blend yielded and increase in recoil. In addition to recoil increases due to higher intrinsic mass loss from incubated amorphous LMW polymer, it can be speculated that re-modeling of the torsional stresses built up during deployment and subsequent diameter reduction during incubation also leads to a higher degradation rate, thus higher recoil.

Barragan et al showed that the rate of restenosis could be correlated to the elastic recoil of the coronary stents [70, 71]. High elastic recoil is thought by many to cause thrombus formation.

4.5 Degradation Characteristics.

The collapse pressure resistance (scaffolding function) and drug release kinetics (in case of a drug loaded stent) depend on the mass-loss and molecular weight reduction kinetics of the polymer. Hydrolysis is a common method of degradation of polymers. PLLA degrades by hydrolysis, and its degradation products can be metabolized. PLLA degrades to lactic acid via hydrolytic de-esterification, and lactic acid forms pyruvate by lactate dehydrogenase action. Pyruvate is an intermediate product in metabolic pathways. It can generate glucose via gluconeogenesis or be metabolized to form CO_2 and water by the citric acid cycle [72]. Degradation normally starts with the amorphous regions before proceeding to the crystalline regions of the polymer. The degradation rates of the blends made from LMW-PDLLA:HMW-PLLA are much lower than the LMW-PLLA:LMW-PDLLA blends. The latter is more amorphous, with dispersed chain packing, thus encouraging water penetration. Degradation of crystalline regions of a polymer proceeds more slowly due to inhibition of hydrolysis by the tight ordering of the hydrophobic polymer chains in comparison with degradation of the amorphous region. Based on this principle we can observe that a higher mass loss is reported on all blends during the first 4 weeks. The degradation rate may slow down thereafter as the crystalline region comes to dominate the structure. The HMW-PLLA shows a plateau after 2 weeks and then proceeds via a very low degradation rate. This to a certain extent conflicts with the higher HMW-PLLA degradation rate results at 37 deg C shown by Satasiya (reproduced in Fig 4.3).

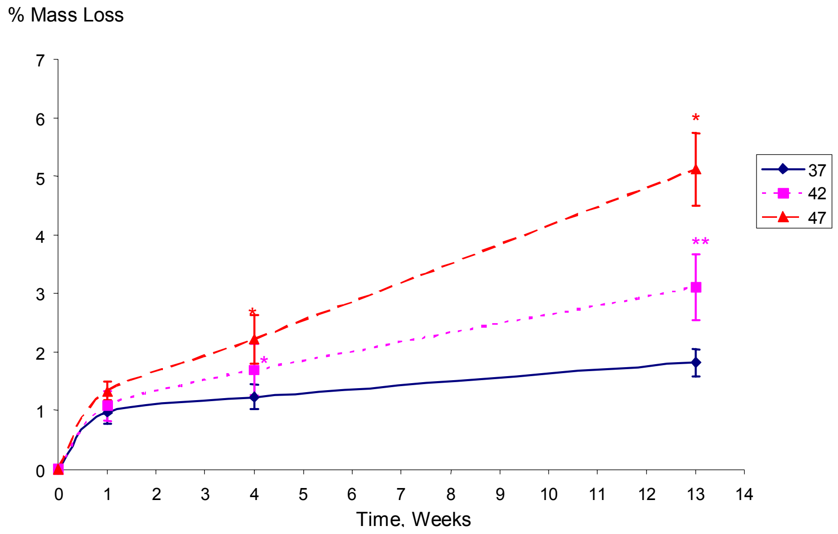


Figure 4.4 HMW-PLLA mass loss rates at various fiber incubation temperatures. Taken from Satasiya [2], with permission.

The kinetics results compare well; degradation rates were observed to be high initially, and gradually, to reduce. This variation may be due to the processing conditions, extrusion methodology, contaminants and temperature of the oven. I strongly believe in this case the oven temperatures regulated the degradation rate due to the method that was adopted in this study. This study was done for a shorter period of time as compared to the 14-week study done by Satasiya [2]. It could be that the total mass loss reached by the end of 6 to 7 months could be similar in both cases due to similar characteristics shown by both. pH is maintained since acidic nature of the medium can induce a bulk hydrolysis and enhance the degradation rate. The mechanical strength of LMW-PDLLA and LMW-PLLA was reduced significantly, which was evident from the breaking of the fibers immersed in the PBS solution. It implies that strong interaction was absent between the L and the D chains, which is a critical part in maintaining the mechanical strength between stereocomplexes. [60].

The average four-week weight change of the HWM-PLLA:LMW-PDLLA blend is low (12%) as compared to the LMW-PLLA:LMW-PDLLA blend (21%). Degradation tests performed on the HMW and LMW PLLA polymers revealed relatively slower degradation rate. The 40:60 blends showed an increase in the degradation rate by 9 % in comparison with the HMW-PLLA:LMW-PDLLA blends, due to a higher percentage of low molecular weight polymer. From the thermal analysis we noticed that blending of high and low molecular weight polymers enhanced the amorphous region while maintaining strong interaction between the different chains. This increased amorphous region was provided mainly by the low molecular weight compounds in the blend, thus accelerating the degradation rate in comparison with HMW PLLA drawn fiber, with a relatively low amorphous content. DSC and Instron instrumentation were not available in time to immediately obtain the shifts in the molecular weight and mechanically test the fibers. These experiments can be repeated and the measurements can be taken confirming the said conclusions. I believe I am correct in saying that by personal experience, the mechanical strength is reduced to a relatively large extent (for a stereocomplex), by the increase in low molecular weight compound content.

CHAPTER 5

CONCLUSIONS

1. Polymeric blends were designed in order to reduce the melting temperature of high molecular weight PLLA and to improve the incorporation of the small molecule drugs. The blends included compositions of HMW PLLA and LMW PDLLA with varying percentages. The blends showed good mechanical characteristics and faster degradation, especially for those blended polymers containing LMW PDLLA.
2. Mechanical testing (uniaxial tension) of fibers made from HMW PLLA and LMW PDLLA blends had characteristics similar to Su's HMW PLLA fiber
3. There was no significant drop in the collapse pressure resistance for stents produced with HMW PLLA-LMW PDLLA fibers after 4 weeks of degradation as compared to the collapse pressures of HMW PLLA stents after 4 weeks of degradation.
4. HMW PLLA-LMW PDLLA and LMW PLLA and LMW PDLLA blends had lower melting points than HMW PLLA. The reduced melting point was sufficient to permit melt extrusion of curcumin-loaded fibers.
5. A first curcumin-loaded stent was produced from the HMW PLLA fiber.
6. Low molecular weight glue was developed to reduce the string formation at weld fillets. This glue was tested for wetting of the surfaces, ease of application and solvent corrosion. Stents fabricated with this glue had good weld integrity upon inflation and

good pressure-induced collapse resistance. This glue can be used as a substitute for the original chloroform/PLLA based glue.

7. Built-up stresses in fibers during stent expansion, as explained by Welch, lead to "twisting" which can affect a triangular cross sectional configuration of the stent rather than a circular one. Removal of fiber twisting and the corresponding stent triangular configuration was demonstrated, using both thermal and mechanical annealing.
8. If PLLA blends are not stored in a controlled atmosphere, then small amounts of moisture in air can degrade the polymer amorphous regions, leading to an increase in melt temperature and glass transition temperature due to the increasing proportion of crystalline polymer.

CHAPTER 6

RECOMMENDATIONS FOR FUTURE WORK

I would like to honestly express some serious recommendations to any other student who wishes to undertake the stent development project. The path to attain a good stent on which experimentation can be done is not easy. It takes atleast two semesters before one can start making stents with good characteristics. We also have to be careful in making conclusions, since they may not tally up with those of other student work.

One significant change that I recommend is to improve the manufacturing techniques for the inner coil biodegradable stents. A better jig design needs to be developed, one that provides an even distribution of tension and pitch during the winding of the polymeric fibers. My experience has led me to believe that consistency and repeatability are very difficult to obtain, contrary to what Su et al [2] has reported. There is a lot of room for improvement in each and every step of fabrication.

From the work I have initiated, it is clear that extensive analysis needs to be done in characterizing PLLA: PDLLA blends. Variations in respect to the percentages of mixture, degradation, inflammatory reaction, need to be done. One can aggressively follow the development of stents based on these blends since they offer similar characteristics to PLLA at the same time providing room for incorporating large quantities of drug. A raw analysis was done to obtain drug release profiles based on the degradation rate by using the equipment available at the time of research. HPLC analysis and other drug distribution studies should also be done.

My internship at Boston Scientific leads me to believe that certain methods and techniques can be adapted to the current stent for better biocompatibility. Coating the stents with a variety of commercially available metals, or composites, can reduce the "bio-incompatibility". I would also recommend an attempt to synthesize the polymers in-house, since it will give the researchers more control of a variety of parameters such as degradation rate, surface energy, surface chemistry, drug loading and manufacturing ease. I also highly recommend continuous interaction and one-on-one meetings with Dr Eberhart in order to brain-storm ideas. We have the best intellectual and resourceful individual working with us, and it would indeed be unwise not to learn from his tremendous experience.

On the final note, all students have done a great effort with the resources at hand. I have pointed out some problems in work done by previous students and like-wise I hope someone can build on what I have started and criticize my efforts, since it is this critical evaluation that will lead to better and exceptional work in the lab.

APPENDIX A

EXTRUSION PROCEDURE

The batch extruder settings in the lab are sub-optimal. It is up to the user to develop and tweak them to the optimal conditions. Following are the steps that are vital to a good extrusion, in my experience.

1) Calibrate the thermostat. Normally there is a variation of 10 deg C, due to contact resistance changes, etc. This has to be done prior to <u>all</u> extrusions so as to have a good handle on temperature settings. It has been noticed that the machine shows 10 deg C lower that what it is set at and what it records. One way of measuring is to use ice cubes and measure the temperature changes in the thermostat and see if it reaches 0 deg C. It may show -10 deg C.
2) The ram and the cylinder have to be cleaned of <u>all</u> debris from the previous extrusion. The smallest particle may distort the movement of the ram, making it difficult to get uniformly thick fiber; it may also result in the jamming of the ram in the cylinder, and permanent distortion of the assembly.
3) During the cleaning process, plenty to acetone should be used to check for debris. The stuck polymer will become white in color and easier to see with acetone application. Using a non-scoring plastic scraper, or a wire brush attached to a drill, the ram and the cylinder have to be cleaned until they become sparkling clean. One way to check for cleanliness is to pass the ram through the cylinder.

   The design tolerances are such that the ram will move slowly through the cylinder (in the vertical position), and come out the other end at a slow pace. If it is held up, this indicates the presence of debris or other blockage, e.g., distortion or scoring.

4) After two runs the filters on the die should be replaced. The die should be screwed on to the cylinder using copper coating on the screws. This will prevent heating and over-expansion of the screws when subjected to high temperature.
5) The polymer should be poured to about $3/4^{th}$ of the cylinder height; the ram should then be placed on top, gradually and carefully. The ram should be tested for circular motion at all times, manually.
6) Using a hydraulic jack the ram should be pressed into the cylinder so that trapped air will escape and the resin packed. At the same time, and at all times, one should check for the circular motion of the ram.
7) Once the contents are tightly packed, the ram should be attached to the cylinder with the provided a nut, which prevents leaks and stabilizes the ram trajectory. The nut should be made "just tight enough" while continuing to check for the circular motion of the ram. If during any of the mentioned steps the ram circular movement ceases, the nut should be loosened. This should be the only explanation for rotary movement failure. If, in spite of this, the ram fails to rotate, then the entire process should be repeated. Otherwise the polymer will burn, and leak out, and the extrusion will be failure.
8) Once the cylinder-ram assembly is placed in the encasing heater, one must ensure that the cylinder ram and the actuator ram are aligned. If they are not aligned, packing should be added differentially at the base of the cylinder so as to change

the angle of the cylinder-ram assembly, so as to axially align it with the actuator ram. Failure to do so will lead to permanent distortion of the assembly and require an expensive and time-consuming repair. Following the alignment steps, tighten the bolts and initiate heating by adjusting the thermostat to the desired temperature.

9) The ram will move down once the polymer melts. Set the actuator speed to about 1.9-3.0 depending on what thickness is desired. Faster speeds will cause thicker fibers to form. Continually monitor the fiber thickness, visually, and toggle the speed to obtain the desired thickness. Start the rear fan to cool the extruded fiber.
10) Once the entire polymer load is extruded, remove all the parts and put them in a high temperature heater at about 400 Deg C except the cylinder, for at least 8 hours. Place the cylinder on a vise (gently!) and let it cool down for a day.

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