Biocompatibility of PLGA-PEG-PLGA Hydrogels, a Short Review

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1.0 Introduction

The poly(lactic-co-glycolic acid) referred to as PLGA is one of the main bioresorbable polymers used in medicine. Initially, bioresorbable polymers were used for surgical sutures, they have since been used as other applications such as implants to repair fractures and drug release devices (Lang, et al.). The PLGA first copolymer was made in the 1970's as was under the trade name of Vicril® which is a synthetic absorbable suture coated with a lactide and glycolide copolymer manufactured by Ethicon Inc of Johnson and Johnson.

The PLGA polymer, is synthesized through random ring-opening copolymerization of the cyclic dimers of glycolic acid and lactic acid. Consecutive monomeric units are linked together by ester bonds. PLGA is soluble in diverse solvents including acetone, ethyl acetate, and chlorinated solvents. PLGA biodegradability can be manipulated by altering its composition; the higher the glycolide content and/or the lower the molar mass, the faster the degradation rate. Therefore, it is possible to synthesize materials with degradation times ranging from weeks to years (Lee, et al., Yasukawa, et al, Manickavasagam, et al)

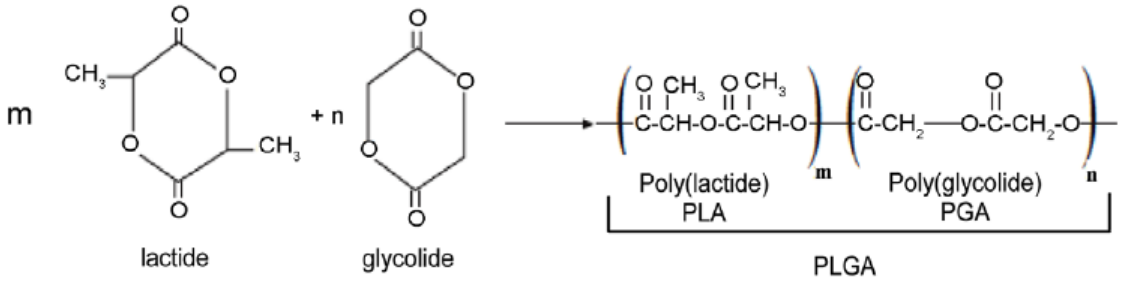
Polymers derived from lactic and glycolic acids have received attention in the research on alternative biodegradable polymers, as they have already received approval from the Food and Drug Administration (FDA) for use as drug release systems. Several studies have been published demonstrating their low toxicity (Stevanovic et al(2009a), Stevanvoic, et al(2009b), and Erbetta et al.)

A major interest in the use of the PLGA polymers is is ability to control both the mechanical and degradation time properties through monomer ratios (Jain). The copolymers can present linear and saturated chains, a constant rate of biodegradation, mechanical resistance, crystallinity, hydrophobicity, regular geometry of individual chains, and thermoplastic behavior. They possess an asymmetric center, presented by the lactic acid (PLA) in a racemic mixture (D,L-PLA) connected to the glycolic acid polymer (PGA).

Figure 1 shows the individual reagents lactide and glycolide and the subsequent repeating poly(lactide) and poly(glycolide) repeating units comprising the PLGA polymer.

Figure 1 Chemical Structure of lactide and glycolide

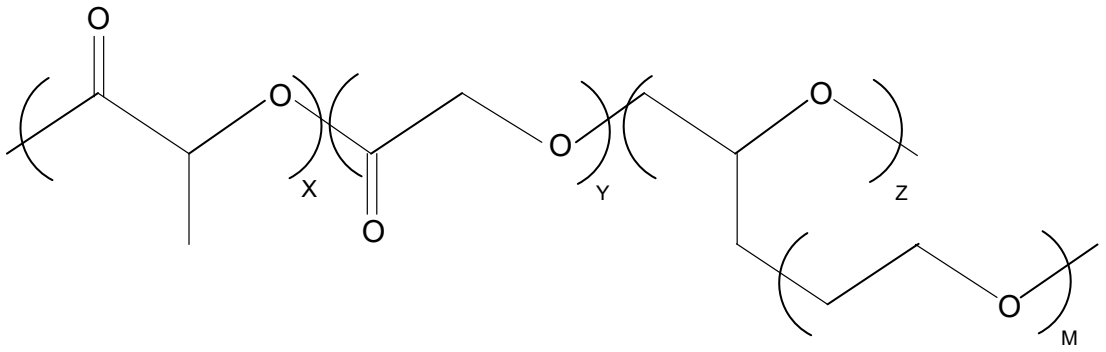
The ring opening step is not shown. The resulting polymer with the subscripts "m" and "n" refer to repeating units from the lactide and glycolide starting reagents.



The current polymer being used by Vivos was formulated by PNNL. Figure 2 shows the schematic of the polymeric components. The PLGA-*g*-PEG polymers controlled the degree of PEG graft incorporation by the amount of PEG in the feed solutions and the reaction temperature (which also varied the polymer molecular weight). The gelation temperatures increased with increasing PEG content. Therefore, the polymer concentration and molecular weight also affected gelation temperatures. Gelation temperatures were obtained from 15ºC to 34ºC. The LA/GA/EG compositions for a 34.3ºC gelation was 3.30/1/3.70 (Tarasevich, et al.)

Figure 2. Schematic Drawing of the PLGA-g-PEG Polymer

The X, Y, Z, and M indicates the number of repeat units of LA, GA, number of PEG graphs, and the number of repeat units of EG in PEG graft, respectively (Tarasevich, et al.). Note the epoxy terminated PEG (EPEG) is grafted on the monomethoxy polyethylene glycol polymer (MPEG).

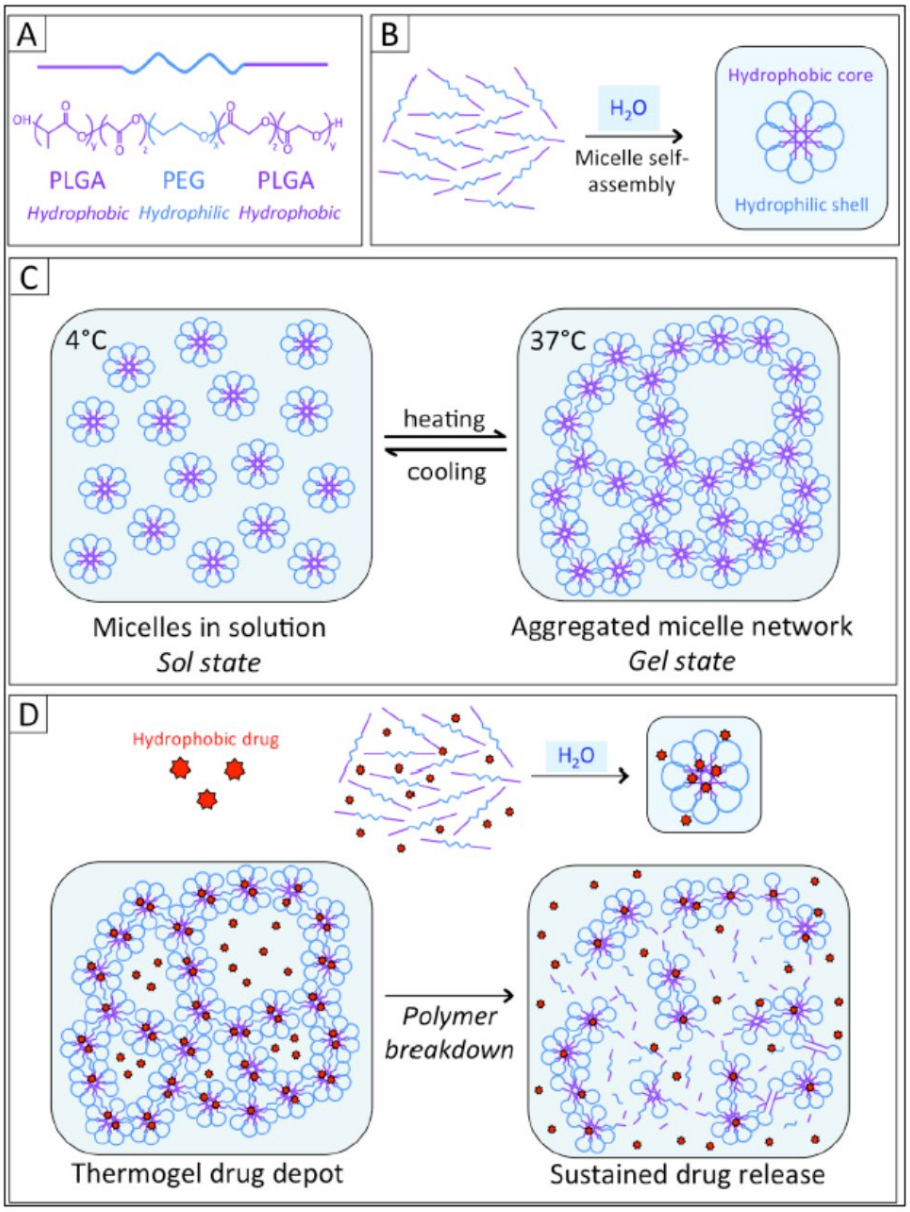


Since the PNNL formulation, Vivos has contracted with Akina, Inc of Layfette, IN for "fine tuning" the formula and synthesis. The Akina formulation has been adopted in the Vivos raw material (RM-013, PLGA-*g*-PEG) product.

As to how a tri-block polymer becomes a polymer carrier, a conceptual approach is shown in Figure 3, from synthesis (A); micelle self-assembly (B); in the sol-gel state (C); mixed with a drug for delivery and subsequent breakdown of the hydrogel (D) (Gervais).

Note that the drawing in B when the polymer is mixed with water is analogous to the Vivos polymer in solution with sterile phosphate buffered saline.

Figure 3 Tri-Block Thermogel Polymer

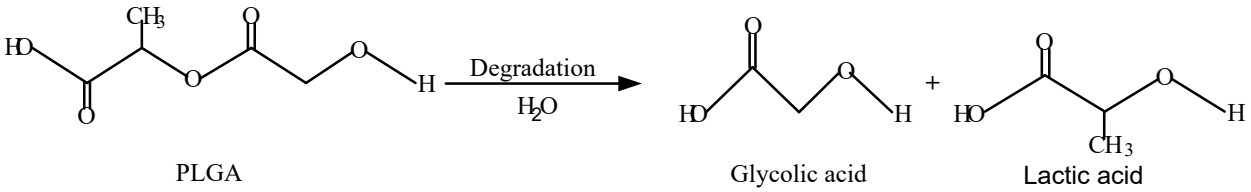


A. Individual polymer subunit structure. **B**. When placed in presence of water, individual polymer subunits self-assemble into micelles with hydrophobic PLGA cores and hydrophilic PEG shells. **C**. At low temperature, micelles remain in aqueous solution. As the temperature is raised, cross-link formation between micelles results in formation of a three-dimensional aggregated micelle network. **D**. Hydrophobic drugs can be incorporated into the micelle core and thus solubilized in the polymer solution. Hydrolytic degradation of the polymer network results in slow sustained release of drug from the micelle cores (Gervais).

2.0 Degradation of PLGA

The PLGA hydrogel is a biocompatible polymer and is adsorbed into the cellular biosynthetic pathways. A biodegradation pathway was proposed by Garcia-Estrada as a hydrolytic function. The hydrolysis of PLGA yields glycolic acid and lactic acid, Figure 4.(Garcia-Estrada, et al.).

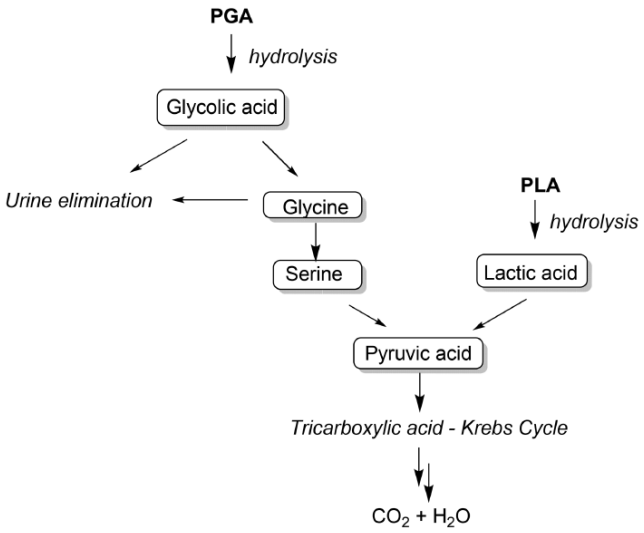
Figure 4 Degradation of PLGA



Laycock, et al, reported that the degradation of polylactide (PLA) and polyglycolide (PGA) mainly occurs by bulk hydrolysis, and the hydrolysis rate is lowered by the crystallinity. The lactic acid and glycolic acid are produced by the hydrolysis of PLA and PGA, respectively. The degradation products are finally incorporated into the Krebs cycle (Figure 5) and are ultimately expired as CO_2 (Pappalardo, et. al)

Notably, the bulk degradation of both PLA and PGA does not immediately produce a decreasing of the mass of the implant, which is instead delayed to days, months or years, until the molecular weight of the polymeric chains is reduced to such extent that allows them to freely diffuse out from the polymer matrix. At this point, the surrounding tissue may not able to eliminate the acidic byproducts of a rapidly degrading implant. Thus, an inflammatory or adverse response may result due to a decrease in the microenvironment pH levels. To overcome this problem, the incorporation of basic salts to prevent a drop in the pH during the degradation process was shown to be successful in vitro (Agrawal, et al.).

Figure 5 Degradation Pathways for Polylactide and Polyglycolide

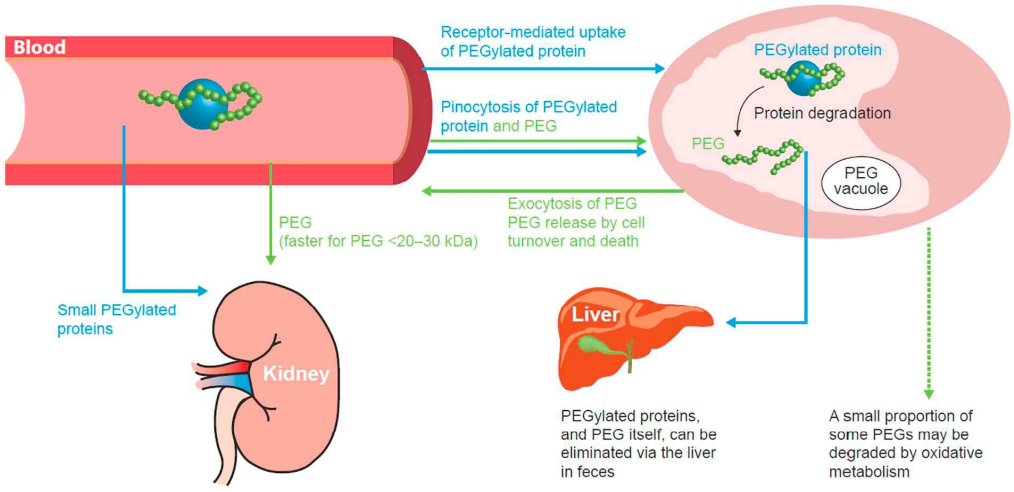


3.0 Degradation of PEG

Molecules of PEG have a simple, repetitive structure and are chemically inert with low toxicity. The linear structure is expressed as $H\text{-}(O\text{-}CH_2\text{-}CH_2)_n\text{-}OH$.

They are uncharged, water soluble, nonreactive, and without any specific receptors or targets in the body (Schellekens 2013; Webster et al., 2007). Accumulation of large PEG molecules (> 20–30 kDa) remains a possible concern because of their increasingly low clearance with higher molecular size (Yamaoka et al., 1994). Cellular vacuolation in certain tissues and cell types, which has been observed in nonclinical toxicology studies for ~50% of approved PEGylated drugs, is the only effect attributed to PEG thus far (Baumann et al., 2014; Ivens et al., 2015). The pharmacokinetic properties of PEGylated proteins are initially driven by the two major parts of the molecule: the protein itself and its conjugated PEG. When the PEG remains after protein catabolism, its biodistribution and pharmacokinetic properties are governed by PEG-related mechanisms, which are outlined in Figure 6 (Baumann et al., 2014).

Figure 6 Distribution and Excretion of PEG and PEGylated Proteins



Bauman et al (2019) concluded that following single-dose administration of the 60-kDa PEG component of BAY 94-9027 ([prop-^{14}C]BAY 1025662) to male rats, which approximated the PEG dose that patients would receive with 30 years of BAY 94-9027 treatment, several conclusions can be drawn. Primarily, results demonstrated that excretion processes are in place for higher molecular weight PEGs, such as the PEG-60 moiety used in BAY 94-9027. Large PEG molecules (≥20 kDa) are continuously but slowly eliminated, resulting in long elimination half-lives in organs and tissues; thus, PEG accumulates with repeated dosing until a steady state is reached. Once a steady state is reached, the PEG concentration in plasma, organs, and tissues remains constant with no further accumulation.

NOTE: the MPEG and EPEG used in the Vivos' hydrogel is 750 Daltons and 500 Daltons, respectively. From the above referenced journal articles, the lower Da PEGs would be cleared quickly from mammalian patients.

4.0 Compatibility of PLGA-PEG Polymers

4.1 Animal Studies

Several laboratory investigations using PLGA-based devices are reported in the literature, evidencing the biocompatibility and biodegradability of this polymer. Souza et al performed a study on New Zealand white rabbits using Tacrolimus-loaded PLGA implants (TC-PLGA). The use of these devices showed no abnormalities during the study, and no signs of inflammation or any retinal hemorrhage or detachments. Also, ophthalmic examination revealed no evidence of toxic effects during the six weeks of the trial, in which 99.997% of the drug was released. Peng et al. evaluated the biocompatibility and biodegradability of PLGA 50/50 (i.e., 50% PLA, 50% PGA) microfilms used as subconjunctival implants in New Zealand white rabbits. An *in vitro*

degradation test showed a directly proportional increase of water absorption and immersion time after a week, which accelerates hydrolysis and leads to substantial decrease in the molar mass, thus accelerating degradation of the polymer. In addition, an *in vivo* study Peng, et al., demonstrated the biocompatibility and non-toxicity of the implant since no evidence of inflammation, fibrosis, or scarring was evident (Souza et. al, Peng et. al).

Summer, et al, reported on the LD levels of glycolic and lactic acid for rat, guinea pig, mouse, and rabbit, Table 2.

Table 1 Toxological Evaluation of Glycolic Acid and of Lactic Acid

Compound	Species	Application[a]	LD mg/kg	
glycolic acid	rat	oral	LD_{50}	1950
glycolic acid	rat	intravenous	LD_{50}	1000
glycolic acid	guinea pig	oral	LD_{50}	1920
D,L-lactic acid	rat	oral, s.c.	LD_{50}	3730
D,L-lactic acid	rat	i.p.	LD_{50}	2000
D,L-lactic acid	mouse	oral	LD_{50}	4875
D,L-lactic acid	mouse	s.c.	LD_{50}	4875
D,L-lactic acid	rabbit	oral	LD_{low}	500
D,L-lactic acid	guinea pig	oral	LD_{50}	1820
[a] s.c. = subcutaneous; i.p. = intraperitoneal				

The acute toxicity of glycolic acid is low, and it is present as a metabolite in the human body. This low toxicity is further reflected by the low toxicity of ethylene glycol since glycolic acid is an important metabolite of ethylene glycol degradation in humans. Also, the acute toxicity of lactic acid is low. Most mammals, including humans tolerate oral doses of greater than 1500 mg/kg (Pappalardo).

Ina study by Ma, et al, degradation and biocompatibility of 20 wt% PLGA-PEG-PLGA hydrogels were reported. For the *in vitro* degradation study, the hydrogels were incubated in phosphate buffered saline. Approximately 70% of the hydrogel degraded in 40 days (Figure 7). The *in vivo* biodegradation and biocompatibility of the PLGA-PEG-PLGA hydrogels (20 wt%) were subcutaneously injected into rats. The gels degraded gradually in the subcutaneous layer in 4 weeks and completely disappeared after 5 weeks (Figure 8).

The hydrogels displayed faster degradation *in vivo* than *in vitro*. The possible reasons are that cells and enzymes in the subcutaneous layer accelerate the degradation processes. Also, the accumulation of degradation intermediates in the subcutaneous layer may produce a slightly acid environment which also accelerate the biodegradation (Ma et al).

Figure 7 In vitro study of the hydrogel (20 wt%) degradation incubated in phosphate buffered saline.

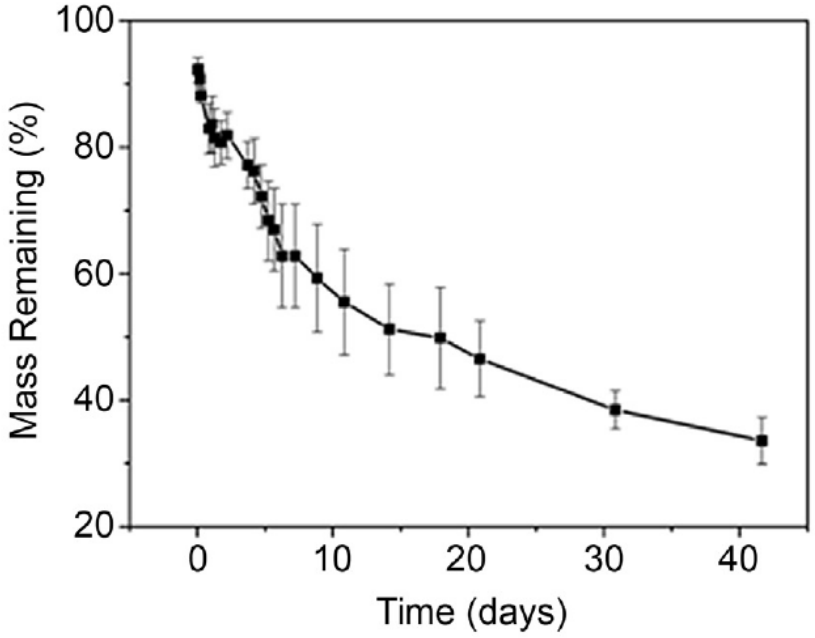


Figure 8 . Hydrogel degradation over time from subcutaneous injects in Wistar rats.

Figure 8 shows the in vivo gel maintenance of PLGA-PEG-PLGA hydrogels (0.5 mL, 20 wt%). The photographs were taken at 10 minutes (0 D), 7. 14. And 35 days post subcutaneous injection.

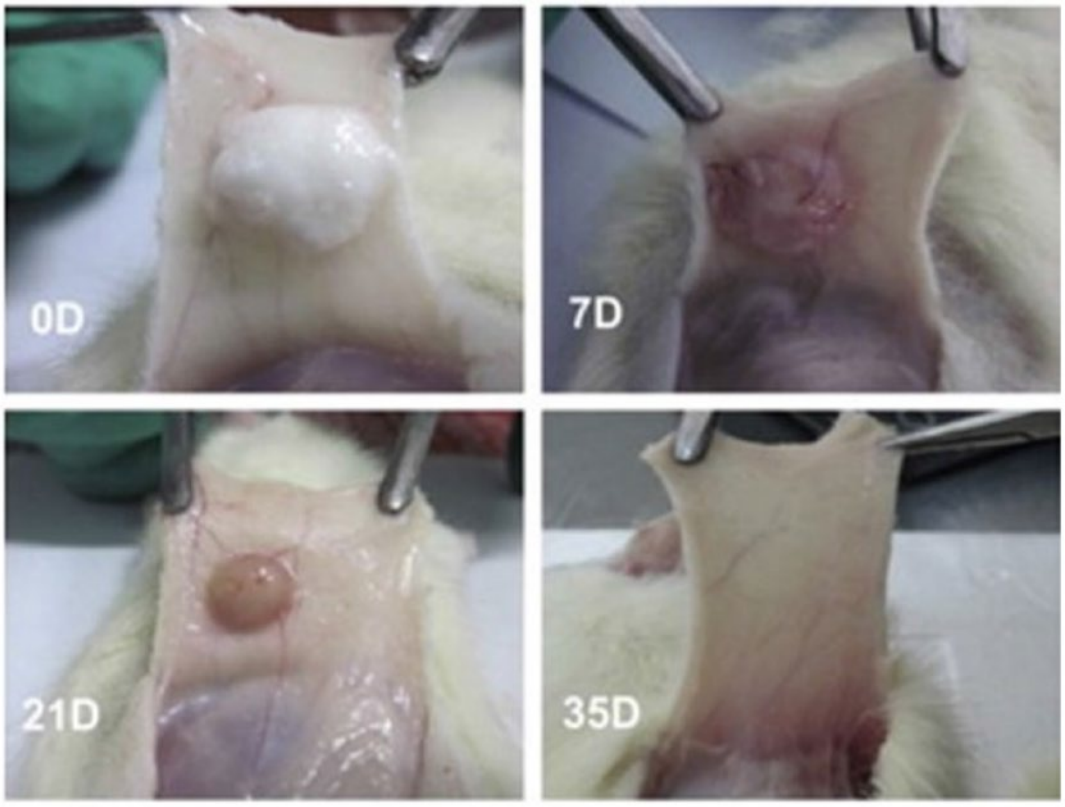


Figure 9 Indicates the Associated Histological Interrogation of the Tissues Surrounding the Hydrogel Injected Area.

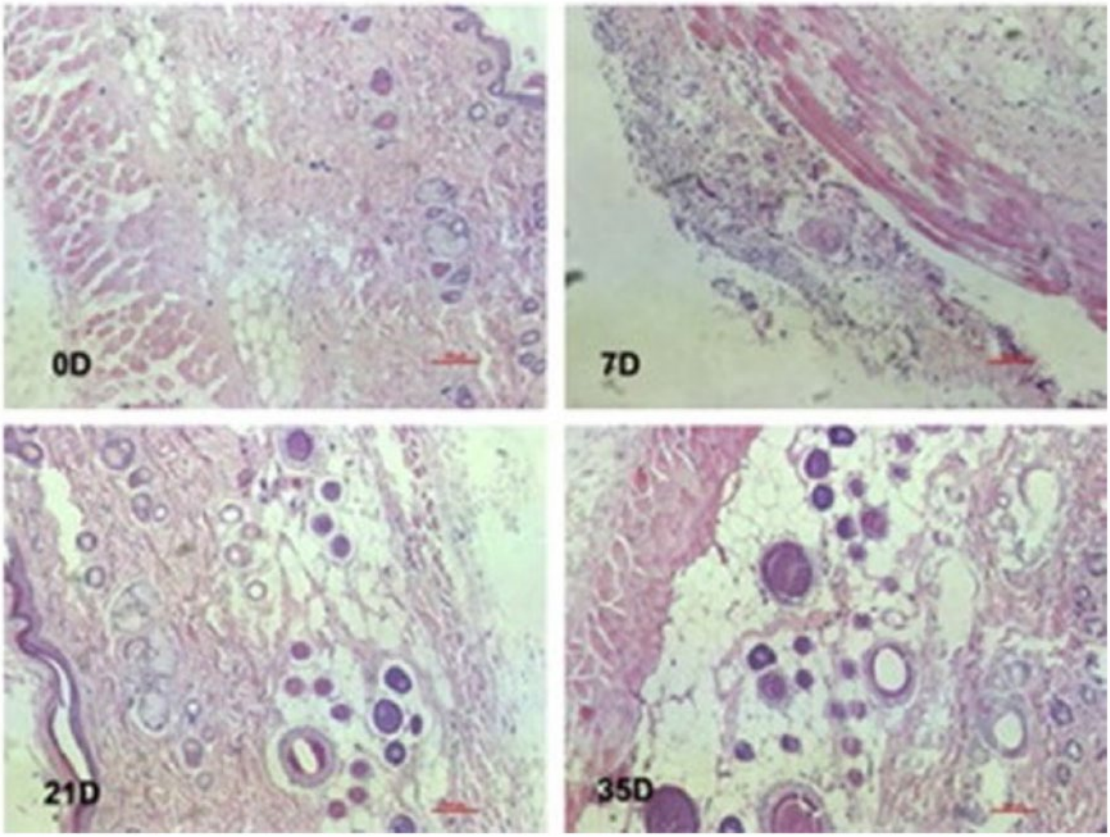


Figure 9 time periods are associated with those shown in Figure 8. The histological analysis (hematoxylin and eosin stained) indicated an absence of necrosis, edema, hemorrhaging and hyperemia in all tissue sections. Day 7 does indicate a mild acute inflammation with enhanced neutrophils. However, the inflammation was reduced and markedly and gradually turned into a weak chronic inflammation as the hydrogels gradually degraded. After degradation, the surrounding tissue presented a return to normal, suggesting an acceptable biocompatibility of the hydrogels *in vivo*.

5.0 Discussion

PLGA-PEG-PLGA (PPP) triblock copolymer is one of the most widely studies thermosensitive hydrogels owing to its non-toxic, biocompatible, biodegradable, and thermosensitive properties. The PPP thermosensitive hydrogels are investigated and used as *in situ* gels due to the ability to be injected at the target site as a carrier of pharmaceuticals, converted into a gel which remains in place. The different requirements of various therapeutic applications are met by adjusting the properties of the hydrogel. These include sol-gel transition temperature, gel window width, retention time and drug release time (Wang, et. al, 2017).

Furthermore, drug diffusion-based release systems are gaining attention due to cost effectiveness and relatively simple synthesis methodology. Among the various biocompatible polymers, PLGA has attracted attention due to but not limited to:

- Biodegradability and biocompatibility; and
- FDA and European Medicine Agency approval in drug delivery systems (S. Mehta, et. al, 2016).

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