Are *in vivo* gastric bioadhesive forces accurately reflected by *in vitro* experiments?

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ABSTRACT

Bioadhesive polymers have been used in oral drug delivery to prolong the contact of dosage forms with the site of drug absorption. Previous investigators have coated oral dosage forms in polymers that demonstrated bioadhesive properties during *in vitro* screens in efforts to prolong the gastric residence of drugs absorbed only in the stomach and proximal duodenum without clinical success. To further investigate the bioadhesive properties of the gastric environment, an *in vivo* quantitative bioadhesive fracture strength test was developed. Bioadhesive and non-bioadhesive bioerodible polymers with potential for use in oral drug delivery were tested for bioadhesive fracture strength both *in vivo* and *in vitro*. Surprisingly, no statistically significant difference was found between the bioadhesive fracture strength of fast eroding polyanhydride and slowly eroding hydrophobic polymers *in vivo*. When the same polymers were tested *in vitro*, the expected difference was observed. The lack of IVIVC (*in vitro/in vivo* correlation) among bioadhesive fracture strengths reflects the clinical finding that polymers that produced strong bioadhesive forces *in vitro* may not achieve prolonged gastric retention *in vivo* due to differences between the *in vitro* screening conditions and the *in vivo* bioadhesive environment.

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1. Introduction

In the late 1960s and early 1970s, investigators reported the first *in vivo* quantitative tensile bioadhesion measurements of marine invertebrates, particularly limpets, to rocks [4,7,12,14,33]. In most experiments a linear translating motor in series with a load cell separated each limpet, by the shell, from the substrate to which it was anchored [7,14]. The mucus adhering the feet of the limpets to various substrates is extremely bioadhesive, 1.95–5.8 kg/cm^2 [7]. Due to the unique anatomical features of marine invertebrates, namely the shell and the external secretion of strongly bioadhesive mucus, tensile bioadhesion measurements were readily obtained [7].

Mammalian bioadhesion tensile testing results were first reported in 1982 when Marvola et al. made measurements on excised intestines from freshly slaughtered sheep [24]. Martti measured the "detachment force" necessary to separate a pill from various sections of the esophagus and intestines [24]. Force was measured by adding water into a beaker until the weight of the water exceeded the bioadhesive fracture strength [24]. Since that time investigators have employed various materials testing apparatus including tensiometers and microbalances to measure the fracture strength of freshly excised tissues in various states of simulated physiological conditions [1,7,13,17,19–22,24,29–31,35]. Investigators including Mathiowitz et al. have shown a strong correlation between *in vitro* fracture strength and *in vivo* transit time results [2,3,8,15,25,28].

Numerous *in vitro* material testing methods exist for quantifying bioadhesive forces that correlate with the overall goals of bioadhesive drug delivery: to promote intimate contact of a dosage with the gastrointestinal mucosa and extend gastrointestinal residence yielding increased bioavailability of a therapeutic agent [6,18,25,32]. However, most *in vivo* bioadhesion testing involves quantifying parameters associated with the goals of bioadhesion such as residence time or relative bioavailability [2,3,8,9,23,25,28]. We believe that the following work provides the first *in vivo* bioadhesive force measurements and the first direct comparison of bioadhesive forces *in vivo* and *in vitro* using a single testing method.

Medical bioadhesives include any of a class of biomaterials that adhere to biological substrates [25,36]. Polymer bioadhesives are used in many medical devices and drug delivery systems including transdermal patches and Gliadel wafers [25]. To date bioadhesive polymers have not achieved clinically significantly improved gastric retention time [5,11,34]. Numerous therapeutic agents, especially polar and anionic small molecules, would greatly benefit from improved gastric retention time [5,11,34].

In vivo bioadhesion measurements have consisted of transit time or relative bioavailability assays [2,3,8,25,28]. Prevalent methods for monitoring gastrointestinal transit time of radio-opaque or radiation emitting doses include X-ray and gamma scintigraphy [2,3,8,25,28]. Relative bioavailability measurements are made by comparing the plasma level concentrations of drugs administered in bioadhesive per oral dosage forms compared to standard per oral dosage forms and

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intravenous infusions [25]. Each of these methods provides data that support or reject the bioadhesiveness of a material, which can be correlated indirectly to parameters measured *in vitro*.

One major obstacle in screening bioadhesives is the lack of *in vivo* quantitative methodologies that are directly comparable to *in vitro* testing data [2,3,8,15]. We report a novel means of obtaining *in vivo* bioadhesive fracture strength by testing through a surgically implanted, re-closable gastric cannula. Investigating the link between *in vitro* and *in vivo* bioadhesion experiments will lead to improved screening methods for bioadhesive materials and improved translational research outcomes when transitioning from bench top to preclinical trials. Quantitative *in vivo* bioadhesion measurements are useful in establishing if the results obtained *in vitro* reflect the *in vivo* environment. The new technique for comparing *in vivo* to *in vitro* bioadhesion measurements quantitatively provides a means for analyzing the correlation between *in vitro* and *in vivo* bioadhesive performance indicator, fracture strength.

Establishing the criteria that yield an effective bioadhesive *in vivo* and then linking it to *in vitro* data will yield improved understanding of how to design bioadhesive materials for gastroretentive oral drug delivery systems. In this paper we optimized the testing parameters (contact force, contact time, presence of PBS, and testing speed) for poly(fumaric-co-sebacic anhydride), which has demonstrated strong bioadhesive fracture strength in previous studies performed on small intestinal tissue [25,26,29–31]. We then applied the optimized conditions to measure the bioadhesive fracture strengths of five bioerodible polymers *in vivo* and *in vitro*. Within the course of *in vivo* testing a gastric cannula confining apparatus was machined to limit motion of the stomach during testing and to more closely approximate the *in vitro* settings. Based on the results, the bioadhesive fracture strength of the loosely adherent gastric mucus layer was measured *in vitro* to test the hypothesis that the *in vivo* bioadhesive environment is governed primarily by the properties of the loosely adherent mucus.

2. Materials and methods

2.1. Materials selection: bioerodible polymers

Five low melting temperature, bioerodible, thermoplastic polymers that have proven orally acceptable in small animal trials were used throughout the *in vitro* and *in vivo* experiments [9,10,25,26,29–31]. Each polymer, upon introduction into the gastric environment presents a hydrophobic surface. In the presence of water, the polymer chains undergo hydrolysis at the water-labile bonds at varying rates, which increased the hydrogen bonding capacity of the polymers and increasing bioadhesion [25]. Three of the polymers were synthesized in-house, poly(fumaric-co-sebacic anhydride) 20:80 (PFASA2080) M_w=12.5 kDa, poly(Adipic Anhydride) (PAA) M_w= 7.5 kDa, and poly(carboxyphenoxy-co-sebacic anhydride) 20:80 (PCPHSA2080) M_w=10 kDa. The other two polymers tested, poly (caprolactone) (PCL) (Sigma Aldrich Saint Louis, MO) M_w=65 kDa and poly(lactic-co-glycolic acid) 50:50 (PLGA5050) M_w=25 kDa (Resomer 503H, Boehringer-Ingelheim Ingelheim, Germany) were purchased.

PFASA2080 and PAA are fast-eroding anhydride polymers that undergo hydrolysis rapidly to expose carboxylic acid residues rapidly enough to produce hydrogen bonding to mucus during gastrointestinal transit indicating that they would be good bioadhesives [25,26,29–31]. In previous studies FASA2080 has demonstrated strong bioadhesion to intestinal mucus compared to slow eroding hydrophobic polymers (e.g. PCL) by numerous techniques including everted sac, CAHN microbalance, and X-ray transit time [25,26,29–31].

PFASA2080 has been one of the most successful polymers for increasing total gastrointestinal transit time [9]. In a previous investigation by our lab 90% of a population of PFASA2080 microspheres was eliminated after 34 h, while the hydrogel alginate took 20 h [9]. However, the amount of time the microspheres remain in the stomach was not studied.

PCPHSA2080 is an aromatic anhydride polymer and therefore degrades more slowly than aliphatic PAA and PFASA2080 [25]. PCL and PLGA5050 are the slowest eroding polymers of the panel and bond to mucus primarily through hydrophobic–hydrophobic interactions shown in previous studies to be significantly lower in magnitude than more rapidly eroding polyanhydride polymers [25]. We believe the *in vitro* and *in vivo* results are the first reported rat gastric bioadhesion on all of the tested polymers.

2.2. Probe preparation

Each polymer was heated to 90 °C, at least 5 °C above the melting temperature. Stainless steel pins were dipped into the polymer and then allowed to cool suspended head-down to form polymer beads for testing. The diameter of each probe is measured by calipers (Mitutoyo Kawasaki, Japan) and the diameter is used in projected cross-sectional area of probe–tissue contact calculations. Probes range from 1.5–2.5 mm in diameter chosen to ensure they will easily fit through the lumen of the gastric cannula (4.5 mm) during *in vivo* testing. Probe size was chosen based on previous studies in our lab that indicated probes on the order of a millimeter in diameter produce bioadhesive tensile forces detectable by the Texture Analyzer load cell. While not in use probes were stored at −20 °C in vacuum-sealed bags under nitrogen gas in the presence of Drierite (W.A. Hammond Drierite Xenia, OH) desiccant to minimize degradation between manufacture and testing. Each probe was tested only once since contact with the testing buffer accelerates polymer degradation.

2.3. Gastric cannula surgical procedure

The cannula consists of the barrel of a polypropylene 1 cm^3 syringe (Becton Dickinson Franklin Lakes, NJ) that has been machined to remove the dispensing tip and reduce the length to 3/4 in. The inner diameter of the gastric cannula was chosen to easily fit the polymer probes. As a result of the relatively large diameter of the gastric cannula, direct gastric cannulation was required, rather than transesophageal or nasogastric tube placement.

The modified syringe barrel is then tapped to interface with a 10–32 knurled, unslotted stainless steel thumb screw. Two tightly fitting silicone bands, 1 mm thick sections of 1/4 inch OD × 1/8 inch ID Silastic tubing (Cole Palmer Vernon Hills, IL), were fitted tightly around the cannula for anchoring to the stomach serosa and dermis as diagrammed in Fig. 1a.

Each 400–500 g albino Spague–Dawley rats was fasted overnight in a metabolic cage and then induced on 3.5% and maintained at 2.5% isoflurane adjusted to effect. Hair was clipped from the ventral rib cage to the pelvis and from the left shoulder to the left hip and prepared with iodophor to sterilize the skin. The rat was covered in a fenestrated drape and body temperature was maintained on a heating pad set to low. A 3–5 cm incision was made in the skin and ventral mid-line fascia caudal to the xiphoid process. Upon entering the peritoneal cavity, the least vascularized portion of the greater curvature of the fundus was identified. Using 7-0 prolene a purse-string suture was made at the site of least vasculature to minimize blood loss as reported by Pare et al. [27]. Once the purse-string suture was in place, a scalpel armed with a number 11 blade punctured the full thickness of the stomach mucosa within the middle of the purse-string suture. Pressure was applied immediately using sterile gauze to achieve hemostasis.

Afterwards, the flanged finger holds of the syringe that form the base of the cannula was inserted through the puncture site into the stomach. The purse-string was pulled tightly around the cannula and secured. Then a series of 3–5 simple interrupted seromuscular sutures affix the suture cuff to the stomach to minimize movement of the cannula with respect to the stomach.

Once the flanged portion of the cannula has been placed within the stomach, an exit point for the tube portion of the cannula is chosen in the left lateral abdominal oblique muscles and overlying skin. With another