

Tracking of Drug Release and Material Fate for Naturally Derived Omega-3 Fatty Acid Biomaterials

KEITH M. FAUCHER,¹ NATALIE ARTZI,^{2,3} MOSHE BECK,^{2,4} RITA BECKERMAN,^{2,4} GEOFF MOODIE,¹
THERESA ALBERGO,¹ SUZANNE CONROY,¹ ALICIA DALE,¹ SCOTT CORBEIL,¹ PAUL MARTAKOS,¹
and ELAZER R. EDELMAN^{2,5}

¹Department of Research and Development, MAQUET Vascular Systems (formerly Atrium Medical Corporation), 40 Continental Blvd, Merrimack, NH 03054, USA; ²Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA 02139, USA; ³Department of Anesthesiology, Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115, USA; ⁴ORT Braude College, 21982 Karmiel, Israel; and ⁵Cardiovascular Division, Department of Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115, USA

(Received 13 July 2015; accepted 14 October 2015; published online 26 October 2015)

Associate Editor Rebecca Kuntz-Willits oversaw the review of this article.

Abstract—*In vitro* and *in vivo* studies were conducted on omega-3 fatty acid-derived biomaterials to determine their utility as an implantable material for adhesion prevention following soft tissue hernia repair and as a means to allow for the local delivery of antimicrobial or antibiofilm agents. Naturally derived biomaterials offer several advantages over synthetic materials in the field of medical device development. These advantages include enhanced biocompatibility, elimination of risks posed by the presence of toxic catalysts and chemical crosslinking agents, and derivation from renewable resources. Omega-3 fatty acids are readily available from fish and plant sources and can be used to create implantable biomaterials either as a stand-alone device or as a device coating that can be utilized in local drug delivery applications. In-depth characterization of material erosion degradation over time using non-destructive imaging and chemical characterization techniques provided mechanistic insight into material structure: function relationship. This in turn guided rational tailoring of the material based on varying fatty acid composition to control material residence time and hence drug release. These studies demonstrate the utility of omega-3 fatty acid derived biomaterials as an absorbable material for soft tissue hernia repair and drug delivery applications.

Keywords—Fish oil, Omega-3 fatty acids, *In vivo* material tracking, IVIS, Fluorescence, Biomaterials.

Address correspondence to Keith M. Faucher, Department of Research and Development, MAQUET Vascular Systems (formerly Atrium Medical Corporation), 40 Continental Blvd, Merrimack, NH 03054, USA. Natalie Artzi, Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. Electronic mails: keith.faucher@maquet.com and nartzi@mit.edu

Keith M. Faucher and Natalie Artzi contributed equally to this work.

INTRODUCTION

Medical devices used in hernia repair must do so in a biocompatible manner while enhancing tissue ingrowth and integration of the devices into the abdominal wall without adhesion to visceral organs.⁴ Ideally the device should enable delivery of an antimicrobial/antibiofilm agent to prevent colonization of bacteria onto the device during surgical implantation.² Commonly, drug delivery from medical devices involves multi-layered systems that control delivery of entrapped drug by passive diffusion through a polymeric layer rather than controlled by manipulating the underlying material chemical properties.

Hernia repair/soft tissue repair devices can be constructed as a single biomaterial or a composite device assembled with a mesh underlay coupled with a barrier layer/coating.⁴ Biomaterials utilized in these applications can be comprised of absorbable or non-absorbable materials made of synthetic or natural origin.^{4,14} Non-absorbable synthetic materials commonly utilized in these applications include non-erodible synthetic polymers such as polypropylene (PP), expanded polytetrafluoroethylene (ePTFE), and polyethylene terephthalate (PET). Absorbable materials include elastin, collagen, hyaluronic acid, and carboxymethylcellulose.^{4,6} However, despite the utility of using these materials in hernia/soft tissue repair applications, challenges still exist. Some biomaterials can require hydration prior to implantation and can become sticky and more difficult to handle during

surgery. Difficulty with surgical handling of the implanted device can lead to longer surgical procedures and can increase the risk of infection and adhesion formation. Biological responses to these implanted materials can lead to surgical complications that can require additional treatment and re-operation.^{3,6,9} Therefore, there remains a need for the development of new biomaterials that facilitate improved surgical handling for ease of implantation, reduce the formation of surgical adhesions, and allow for the controlled drug delivery of antimicrobial agents to prevent device colonization with bacteria following implantation.

Naturally derived biomaterials made from renewable resources reduce the risk to the patient by eliminating the need to use toxic catalysts and crosslinking agents commonly used in the production of synthetic polymeric materials, which can leach from the device and result in an increased inflammatory response after implantation. Residual leachable chemicals are an important characteristic that must be considered from regulatory, product safety, design and risk perspectives.^{4,10} While devices have been constructed using naturally-derived protein¹² and cellulose¹⁶ materials, devices constructed using oils and phospholipids¹¹ represent an area of further research and opportunity. Omega-3 fatty acids obtained from fish and plant sources contain a mixture of saturated, monounsaturated, and polyunsaturated fatty acids, which can be used in native triglyceride form or converted into an alternate ester form to create crosslinked, higher molecular weight biomaterials with different physical and chemical properties.

We now detail the utility of using naturally derived oils containing omega-3 fatty acids to create cross-linked fatty acid biomaterials (i.e., O3FA biomaterials) for use in soft tissue repair applications with a focus on achieving adequate surgical handling of the material and improving biocompatibility while enabling controlled drug release. We developed crosslinked O3FA Biomaterials that can be implanted in the form of a stand-alone film or a medical device coating on a surgical mesh. These materials can further be used as a vehicle for localized drug delivery due to the ability to alter drug release rate based on the underlying fatty acid chemistry. The degradation rate of O3FA biomaterials will dictate not only its residence time *in vivo*, but also its drug release kinetics.

To further characterize and gain mechanistic insight into the degradation behavior of O3FA biomaterials, several *in vitro* and *in vivo* studies were conducted. These included using an *in vivo* fluorescence imaging system (IVIS) to evaluate the degradation kinetics of stand-alone O3FA biomaterial kinetics both *in vitro* and *in vivo* using fluorescence imaging of tagged O3FA biomaterials. This technique has previously been

applied to PEG:dextran hydrogels materials.¹ Characterization of explanted O3FA biomaterials using FTIR and gas chromatograph (GC) fatty acid profile assay was also conducted. *In vivo* studies of O3FA biomaterials applied onto a polypropylene mesh were also performed in order to evaluate their utility as a biocompatible medical device material in hernia and soft tissue repair.

In a final set of *in vitro* experiments, the ability to create a family of O3FA based materials with distinct degradation kinetics that give rise to different drug release profiles was investigated. We show that the hydrolysis rates of O3FA biomaterials can be tailored by modulating fatty acid composition and ester form. We found that drug release from these materials predominantly results from fatty acid compositional properties rather than passive diffusion and show high potential as absorbable scaffolds for drug delivery.

MATERIALS AND METHODS

Preparation of Fluorescently Labeled Omega-3 Fatty Acid Derived Biomaterial Films

Fish oil was first thermally pre-treated at 93 °C in a bulk form for 17–24 h (i.e., 1–2 L in a round bottom flask) while bubbling oxygen through the material with stirring to pre-oxidize and create polar functional groups (i.e., hydroxyls) in the fish oil. 12-NBD stearate (Avanti Polar Lipids, 12-NBD Stearate 12-*N*-methyl-(7-nitrobenz-2-oxa-1,3-diazo) amino-stearic acid P/N 810110P) was then added into the fish oil at 0.3% (g/g) prior to casting this into a film. This was accomplished by first mixing the NBD into ethanol at a 6.25 mg/mL concentration, followed by a 1.7 mg/mL of partially crosslinked fish oil dissolved in MTBE. These two components were combined in a 55:45 MTBE-partially crosslinked fish oil/ETOH-NBD Stearate ratio and vortexed till the mixture was homogeneous. The solution was then dried for 24 h under vacuum and then the fluorescent O3FA films were cast on PTFE liners against a flat granite surface using 1, 2, and 4 mil casting knife thicknesses. The films were UV treated for 40–80 min (depending on film thickness) and then thermally crosslinked at 93 °C. This was done so that the 12-NBD stearate label would react with the available hydroxyls in the pre-treated oil and thus be incorporated into the fatty acid chains where the crosslinking occurs. Thermal curing was conducted at 93 °C since it is a temperature at which oxidation and crosslinking of the unsaturated bonds in the fatty acids occurs without causing significant degradation of the triglyceride head group ester as seen when heated to temperatures around 200 °C.¹³ This temperature,

93 °C, favors the formation of ester crosslinks due to the evaporation of water from the fish oil during curing (Figs. 6 and 7b). Following this final thermal crosslinking process the films achieved a solid yet flexible physical state. Film thickness was confirmed with drop gauge measurements and fluorophore content and uniformity were examined by IVIS.

Erosion Studies of O3FA Biomaterial Films

In vitro erosion studies were performed using sterilized O3FA biomaterial films at various thicknesses to model the effects of material thickness on mass loss kinetics. The films were constructed with an NBD-stearate fluorescent tag incorporated into the O3FA biomaterial during manufacture as described above. The samples were weighed and then scanned at 500/560 (E_x/E_m) using IVIS (In Vivo Imaging System, Caliper Systems) techniques. They were then secured in a polypropylene mesh envelope, placed inside six well plates, and had 50 μ L of a physiological media (67% Calf Serum, 37% Dubelcco's Minimum Eagle Media) applied. The media mimics the protein, glucose, sodium, potassium, and calcium salt concentration of peritoneal fluid, and 50 μ L volumes were chosen as being representative of the volume of mouse intraperitoneal fluid.⁸ After incubating at 37 °C under gentle agitation for 48 h, the films were weighed and scanned. Fluorescence intensity was determined by calculating the efficiency in a region of interest for each film after subtracting the background signal in each measurement. Data quantification was done using Living Image software. All calculations were done using Excel including the percent loss in fluorescence.

In Vivo Material Absorption Studies of O3FA Biomaterial Films

An *in vivo* study was performed using IVIS techniques to examine the degradation kinetics of O3FA biomaterial films. The sequential *in-life* imaging provided by IVIS minimizes the number of animals consumed and allows for more data points to be taken over time compared with other techniques that require sacrifice and implant excision at each time point. Using an implantation protocol reviewed and approved by MIT's Committee on Animal Care, sterile 7.9 mm film disks were implanted subcutaneously in the dorsal space of the mice (female SKH1-E nude mice, 4–6 weeks old, weighing between 25 and 30 g, 5 mice per group) through a small 0.5 cm skin incision. The size of the pocket accommodated the film with minimal extraneous space to limit film movement. After closure of the incision, the fluorescence of the films was acquired as described above. Isoflurane anesthetized

mice were imaged periodically through the 10 week in-life portion. Excised film samples (at 4 and 10 weeks) were analyzed using Gas Chromatography and FTIR. The fatty acid content of the films were assessed using a GC Fatty Acid Profile Assay with the myristic acid (C14 fatty acid) serving as a unique biomarker as it was not present in significant quantities in local tissue. Overall composition was monitored using FTIR spectral analysis.

FTIR Analysis

The FTIR spectra of explant samples were obtained on a Digilab FTS-4100 Infrared Spectrophotometer from Agilent. The FTIR-ATR spectra were collected using a Specac Goldengate attenuated total reflection (ATR) anvil press accessory equipped with a fixed diamond crystal covering the 4000–700 cm^{-1} spectral range. Each FTIR-ATR spectrum is the average of 64 scans, using air as reference, at 4 cm^{-1} nominal spectral resolution. The data was analyzed with the Resolutions Pro software.

GC Fatty Acid Profile Analysis

Film explant samples were separated from the surrounding peritoneal tissue, weighed, and dried at 37 °C for 4 days. Dried explant samples at 4 and 10 weeks were pooled, converted into fatty acid methyl esters (FAMES) through trans-esterification and their separation by liquid–liquid extraction and compared to non-implanted film controls. Analysis of the FAMES was performed on the Trace Ultra Gas Chromatograph with TriPlus Autosampler and FID detector from Thermo Fischer Scientific with separation using the FAME-WAX[®] Polyethylene glycol (USP G16) phase capillary GC column (30 m length \times 0.32 mm ID \times 0.25 μ m film) from Restek. The column temperature was programmed from 195 to 240 °C at 1.7 °C/min. The injections were performed with split ratio 100:1 and constant flow operating mode at 1 mL/min (helium used as carrier gas). Flame gases were 350 mL air/min, 35 mL hydrogen/min, and 30 mL Makeup/min. The inlet and detector temperatures were both set at 250 °C. The injection volume was 1 μ L.

In Vivo Adhesion Model Testing of O3FA Biomaterials Applied to Polypropylene Mesh

An adhesion model was performed in female New Zealand White rabbits weighing between 3.2 and 4 kg. The rabbits were anesthetized and a midline laparotomy was performed. The cecum and bowel were exteriorized and digital pressure was exerted to create subserosal hemorrhages over all surfaces of the cecum.

The damaged intestine was then lightly abraded with 4" x 4" 4 ply sterile gauze until punctuate bleeding was observed. The cecum and bowel were then returned to their normal anatomic position. A $4 \times 5 \text{ cm}^2$ area of peritoneum was bilaterally removed.

A $30.5 \times 61 \text{ cm}^2$ panel of knitted polypropylene monofilament bare mesh was coated with an O3FA Biomaterial coating created using fish oil at a 100 mg/in^2 target coating load. Due to the size of the device built, the fish oil was thermally treated at $93 \text{ }^\circ\text{C}$ for 38 h to achieve a similar level of cure to *in vitro* prototype samples. $5 \times 7 \text{ cm}^2$ pieces of mesh were cut from the panel and compared to non-coated polypropylene control articles and directly implanted over the excised area of peritoneum. A total of sixteen samples of each type were implanted with each study group, two samples per animal with eight animals per group. Both test and control articles were affixed to the abdominal wall with a Prolene polypropylene suture in a continuous stitching pattern. Animals were implanted bilaterally. Because the implants were in close proximity and the reaction to one mesh type should not be allowed to influence results from another only one type of mesh was used in each animal. The incision was closed in two layers with 3-0 Vicryl. Care was taken not to damage the material at the incision site or injure the bowel.

Adhesion area and severity were assessed visually by the nearest 25% and scored according to Tables 1 and 2. Once the data was collected it was analyzed for statistical significance using the Kruskal–Wallis test. Statistical significance was defined as p value <0.05 .

In Vitro Drug Release Testing on O3FA Biomaterials of Varying Oil Chemistries

Samples were produced using fish oil, flaxseed oil, and fish oil ethyl ester material combinations and cast onto polypropylene mesh substrates. The $2.5 \times 2.5 \text{ cm}^2$ samples were then thermally cured at $93 \text{ }^\circ\text{C}$ for 24 h. Triclosan was dissolved in methanol and dispensed onto the outer surface of O3FA biomaterial coatings produced from fish oil triglycerides, linseed oil triglycerides, and fish oil triglyceride/fish oil ethyl ester blends. The samples were allowed to dry and evaluated at $37 \text{ }^\circ\text{C}$ in a media consisting of 0.2%

Tween 20 in 0.1 M PBS at a pH 8. A pH of 8.0 was chosen to match the pH range measured in the literature for peritoneal fluid.⁵ The amount of drug eluted from the surface as a function of time was determined by HPLC assay. HPLC Assay conditions consisted of a mobile phase of 85:15 methanol:0.2% acetic acid at a flow rate of 1 mL/min, with a Supelcosil LC 18, $250 \times 4.5 \text{ mm}$ column with five micron particles. The column temperature was set to $30 \text{ }^\circ\text{C}$.

RESULTS

In Vitro Erosion Studies of O3FA Biomaterial Films in Simulated Peritoneal Fluid

IVIS studies were conducted to characterize the erosion behavior in simulated peritoneal fluid of fluorescently labeled stand-alone O3FA biomaterial films of various thicknesses constructed from fish oil triglycerides. Figure 1a presents the swelling data for O3FA biomaterial films showing stabilization within 1 h. IVIS measurements were taken during O3FA erosion studies. Figure 1b is a representative scan of the film samples on the second day of the study. The samples show different fluorescent intensity, which is higher in the thicker films. The IVIS measurements showed that the expected differences in the initial rates of the kinetic curves as a function of thickness can be observed where thinner films degrade more quickly than thicker films (Fig. 1c). The fluorescent signal levels for all films regardless of thickness normalized to about 10–15% of the original value at approximately 6 weeks (Fig. 1c). The erosion profile fits a two exponential decay, similar to previous reports,¹ with the first decay representing a rapid burst of unreacted or unbound (i.e., non-crosslinked) material and the second decay presenting the release of bound (i.e., cross-linked) material following hydrolysis. The mass loss and fluorescence signals correlated in this study.

In Vivo O3FA Biomaterial Film Absorption Studies

O3FA biomaterials contain ester bonds in the triglyceride head groups as well as side chain esters that are formed during the crosslinking process. There

TABLE 1. Adhesion area scoring. percentages are percent of mesh covered by adhesion.

0	None (%)
1	1–25
2	26–50
3	51–75
4	76–100

TABLE 2. Adhesion Severity Scoring.

0	No adhesion
1	Mild, easily blunt dissectible adhesions
2	Moderate adhesion; blunt dissectible with aggressive manipulations, does not tear the organ
3	Dense adhesion; non-blunt dissectible (i.e., requires sharp dissection to separate), tears organ when removed

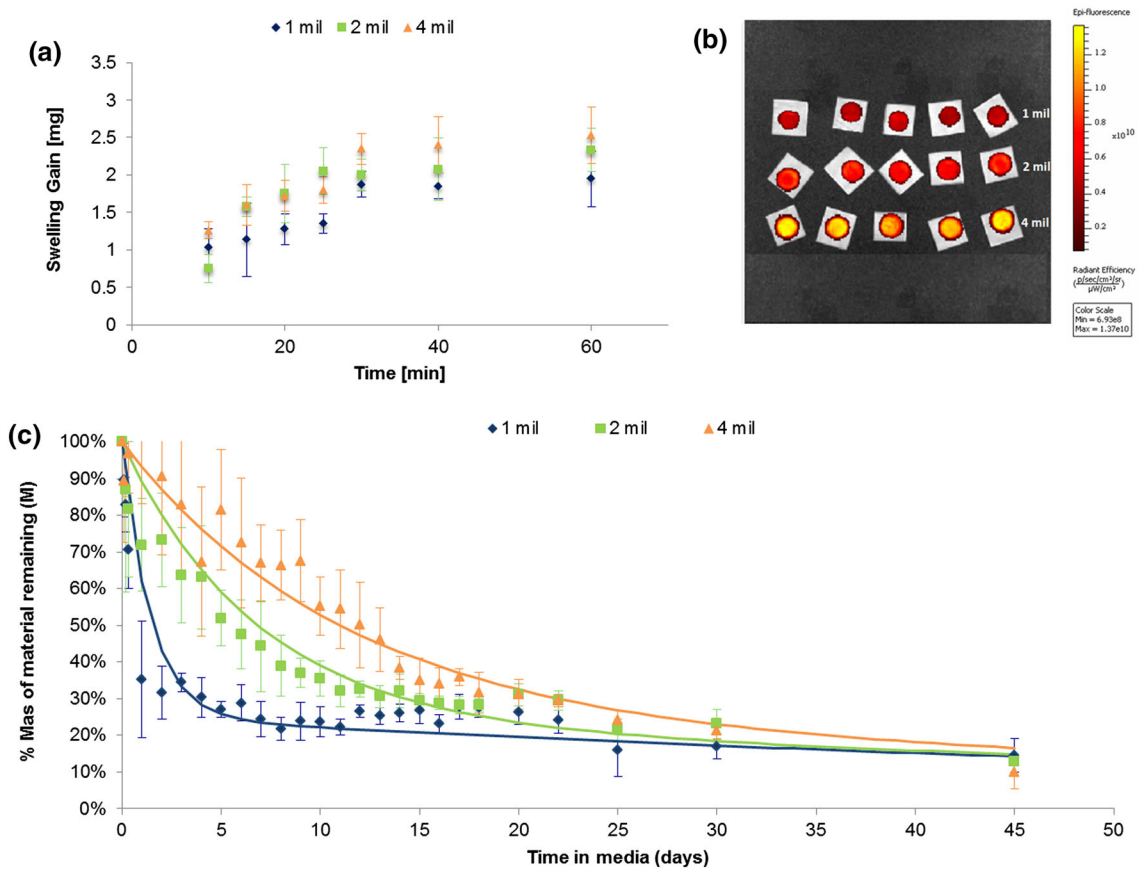


FIGURE 1. *In-vitro* dissolution studies in 67% calf serum, 37% Dubelcco's minimum Eagle media showing the effects of O3FA film biomaterial thickness on (a) Swelling mass gain; (b) Representative measurement scan obtained *via* IVIS of the film samples at day 2; and (c) Material erosion tracked using IVIS and NBD-stearate fluorescence probe.

are distinctive hydrolysis kinetics and differing amounts of the head group and side-chain ester-based populations in O3FA biomaterials. Tracking the specific erosion rates of the side-chain esters and triglyceride head group esters present in the O3FA biomaterials *in vivo* necessitates the use of multiple techniques. Specifically, fluorescence detection with IVIS was used to track the erosion of the side-chain esters and GC fatty acid profile assays were used to monitor the breakdown of the triglyceride head group esters. *In-vivo* imaging monitored the *in situ* erosion kinetics of the side chain esters through the correlation of fluorescence signal loss to labeled ester chains fluorescence loss. Fatty acid profile of explanted films were analyzed by GC using myristic fatty acids (14 carbon chain length, fully saturated) as a marker for the O3FA biomaterial since they are not present in appreciable amounts in tissue, but are present as fatty acids bound to the O3FA biomaterial *via* triglyceride ester bonds. This serves as a measure for the remaining triglyceride esters in the material. The overall chemical

composition of the explanted material was analyzed using FTIR analysis.

IVIS measurements revealed that the majority of labeled side chain esters were degraded at 1 month, regardless of material thickness (Fig. 2a). Figure 2b is a representative IVIS scan taken at the initial time point, of some of the mice that were implanted with the different film samples. The fluorescence probe is seen clearly after implantation. The myristic acid GC assay (Fig. 2c), which represents the triglyceride esters, indicated that at 1 month 50–70% material remained for the various implanted films and at 10 weeks that 30–50% of the material remained *in vivo*. Additionally, explants were dried down and determined to contain a range of 5–25% water at 4 weeks and 16–34% water at 10 weeks. FTIR analysis of the dried films (Fig. 2d) showed that the explanted films spectra had absorptions due to the original implanted fatty acid biomaterial as well as due to amine and amide absorptions. This result is consistent with partial absorption of the implants and correlates with the GC assay data.

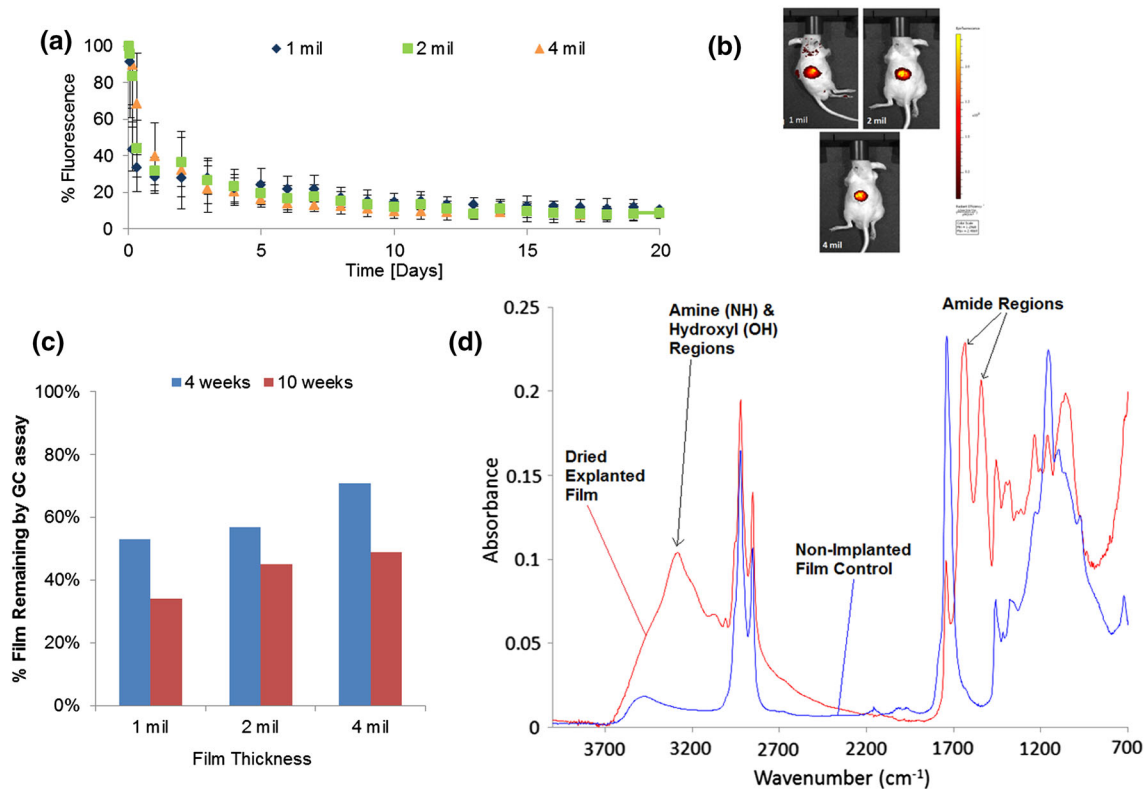


FIGURE 2. *In-vivo* assessment of O3FA biomaterials using (a) *In-situ* IVIS tracking of NBD-stearate label; (b) Representative measurement scan obtained via IVIS at the first time point; (c) Myristic fatty acid gas chromatographic assay of explanted films by GC at 4 and 10 weeks, and (d) FTIR analysis of explanted film after 10 weeks.

In Vivo Evaluation of O3FA Biomaterials Coated onto Mesh in an Adhesion Model

The objective of this study was to evaluate adhesion formation after implantation of bare polypropylene mesh and coated O3FA biomaterial mesh devices in a 28 day rabbit abdominal adhesion model. Bare mesh was compared against O3FA biomaterial coated mesh in terms of adhesion size, adhesion tenacity, and material safety as evaluated by microscopic examination.

Figures 3a and 3b present a comparison of adhesion area and severity for a bare polypropylene mesh and an O3FA biomaterial coated mesh. The adhesion area was significantly smaller and adhesion severity significantly less for O3FA biomaterial coated mesh than bare mesh (both $p < 0.01$). General microscopic evaluation was performed on both samples (Fig. 4a and 4b). Increased numbers of multinucleate giant cells were found around the O3FA biomaterial coated mesh; which is consistent with the presence of an actively eroding coating that is undergoing absorption. Inflammation around the O3FA biomaterial coated sample was mild to moderate and there was good tissue incorporation into the mesh implant. Inflammation around the bare mesh was relatively low, but did show

many more adhesions to the colon. All of these findings for the O3FA biomaterial coated mesh implants are desirable in the design of a biocompatible surgical mesh implant with an absorbable coating.

Fatty Acid Mediated Control of Drug Release Using O3FA Biomaterials

In vitro studies were performed to evaluate the ability to tailor hydrolytic degradation by modifying starting oil material fatty acid composition and fatty acid ester form. *In vivo* IVIS and GC myristic acid assay testing results demonstrated that hydrolysis of the side chain esters occurred much more rapidly than the hydrolysis of the triglyceride esters. It was hypothesized that altering starting oil composition from triglycerides alone to mixing ethyl ester fatty acids with triglyceride oils in different combinations would lead to crosslinking at the hydrocarbon tail, but allow for a faster hydrolysis kinetics due to an overall reduction in crosslinking in neighboring fatty acid chains on the glycerol backbone. This would allow for a faster material hydrolysis rate since the matrix would be dominated by the faster hydrolyzing side chains.

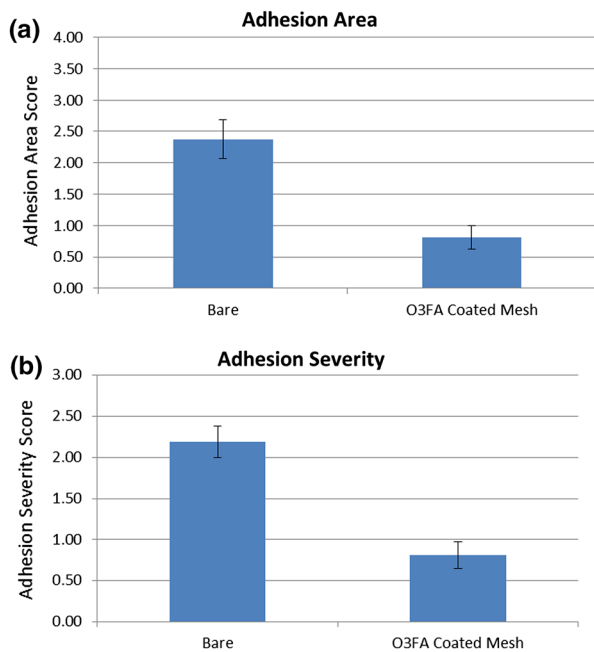


FIGURE 3. Comparison of adhesion (a) area and (b) severity between polypropylene bare mesh control and O3FA biomaterial coated onto polypropylene mesh. O3FA biomaterial values were statistically different from the bare mesh control ($p < 0.01$).

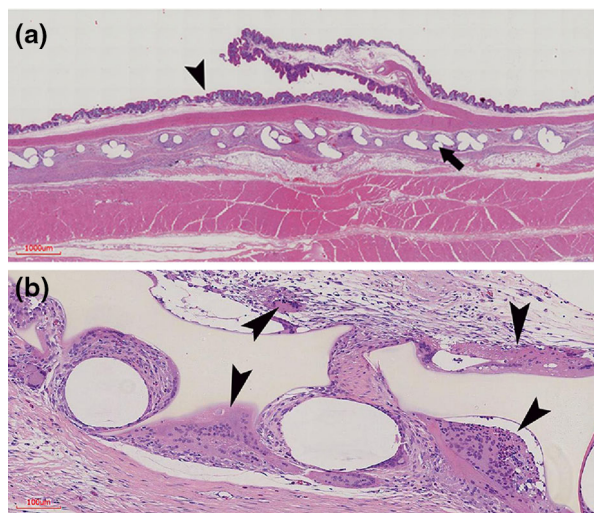


FIGURE 4. Histology stains of explanted (a) polypropylene bare mesh control. Note adhesion of colon (arrowhead) to entire surface of mesh material (arrow), which is also extensively adhered to the abdominal wall (lower half of section). H&E stain. (b) O3FA biomaterial coated onto polypropylene mesh. Arrows indicate foreign body giant cells associated with the coating. Tissue incorporation around the mesh fibers is clearly evident.

The ability to tailor oil fatty acid and ester composition and to alter the underlying O3FA biomaterial chemistry allows for the modulation of release as presented in Fig. 5. O3FA material chemistry was

varied by ester composition (i.e., ethyl ester or triglyceride ester) and/or starting EPA:DHA content in the oil. Fish oil constructs were made of triglyceride esters containing 18% EPA and 12% DHA prior to crosslinking, flaxseed oil constructs were made of triglyceride esters without any EPA or DHA in the material prior to crosslinking, and ethyl ester constructs were made at 90% ethyl esters with 33% EPA, 22% DHA and mixed with 10% fish oil triglycerides.

Triclosan was applied at a $130 \mu\text{g}/\text{in}^2$ onto each construct and then eluted for up to 2 weeks in 0.2% tween, 0.1 M PBS media while maintaining sink conditions (Fig. 5a). Triclosan was chosen as the model antimicrobial compound to highlight the differences in drug release profile between the sample groups since it is commonly used in implantable sutures.¹⁵ The release curves reflected the construct's hydrolysis rates in that the drug is being released very rapidly from the ethyl ester-triglyceride blend. A similar trend release kinetic was observed with the fish oil triglyceride constructs, but the overall release rate was slower. In contrast, the flaxseed oil triglyceride constructs released Triclosan slowly. These results are consistent with a fatty acid mediated drug release rather than the diffusion mediated drug release that is common for most drug eluting medical devices.

DISCUSSION

We examined the potential of O3FA based materials to serve as an absorbable material in soft tissue and hernia repair with the potential of also releasing drug locally with controlled and tunable elution rates. Understanding the formation, erosion and *in vivo* performance of naturally-derived O3FA biomaterials will enable their use in several medical device product applications.

Overview of Omega-3 Fatty Acid Derived Biomaterial Chemistry

The formation of omega-3 fatty acid derived biomaterials is based on a curing process which results in oxidation and the creation of polar functional groups (i.e., carboxyl and hydroxyl) followed by crosslinking (i.e., oxidative crosslinking) (Fig. 6). The materials are produced through thermal oxidative crosslinking without the addition of toxic chemical crosslinking agents or catalysts that can elicit inflammation. Specifically, oils contain a combination of saturated, monounsaturated and polyunsaturated fatty acids, which can be readily oxidized in the presence of heat and/or light with oxygen.⁷ This reaction results in the formation of polar functional groups along the fatty

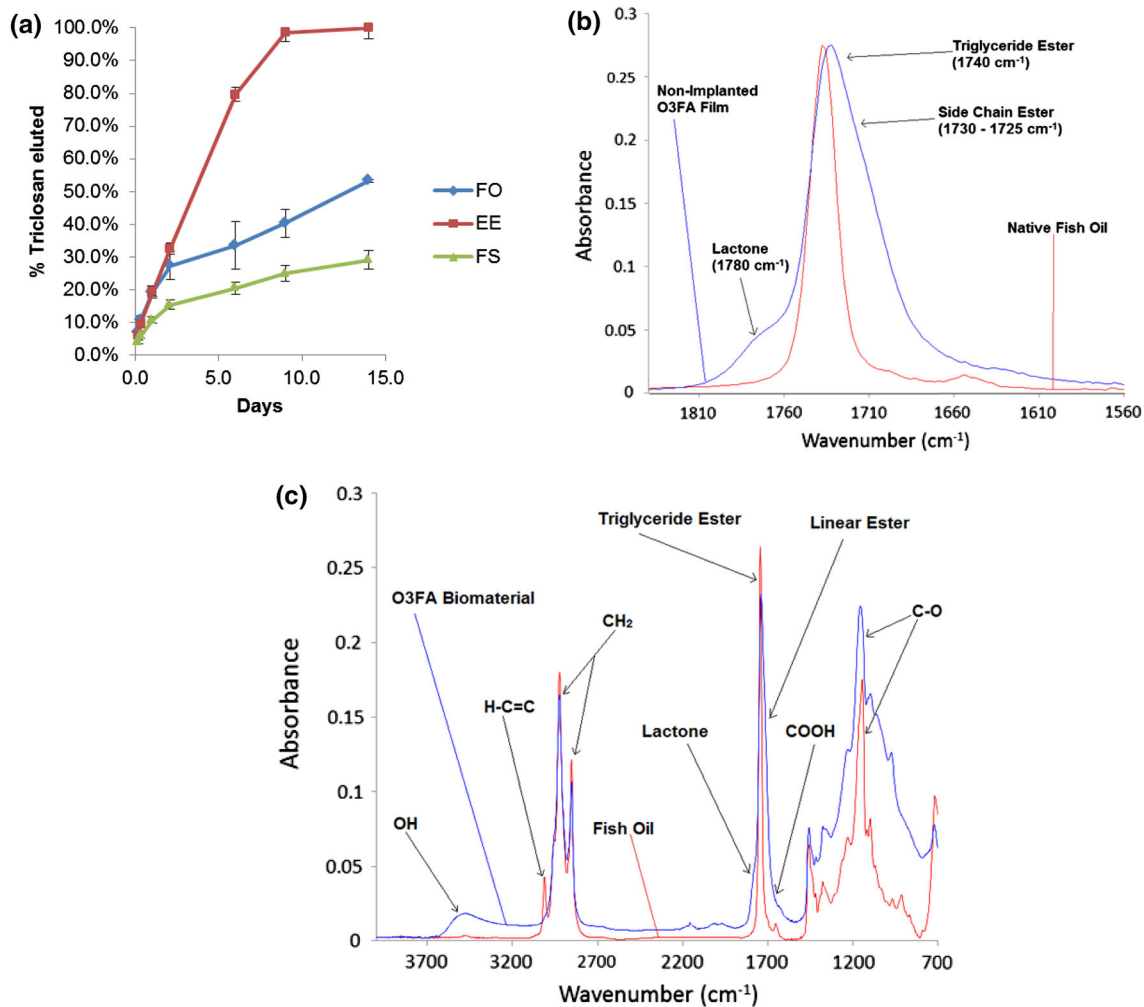


FIGURE 5. (a) *In-vitro* drug release of Triclosan from naturally derived oil materials at a pH 8.0. Data demonstrates that drug release can be controlled based on fatty acid and ester material composition and hydrolysis rate. *FO* 100% fish oil triglycerides, *EE* 90% ethyl esters with 33% EPA, 22% DHA and mixed with 10% fish oil triglycerides, and *FS* 100% Flaxseed oil; (b) FTIR spectral overlay comparing fish oil starting material to O3FA biomaterial highlighting the formation of new esters after the crosslinking process from 1850 to 1560 cm⁻¹, and (c) FTIR spectral overlay comparing fish oil starting material to O3FA biomaterial highlighting the formation of new polar and ester functional groups formed after the crosslinking process from 4000 to 700 cm⁻¹.

acid chains (Fig. 5c). Upon further heating during the process, the oil increases in viscosity due to the release of volatile components (i.e., oxidative byproducts and water) and the formation of new crosslinks between the polar functional groups present in the oil (Fig. 6). The saturated fatty acids are not reactive in this process, but assist in allowing for the cured fish oil to remain flexible by interrupting the crosslink density between the unsaturated fatty acid chains, thus acting as a plasticizing agent. Physically, this process results in the conversion of the liquid oil into a solid, yet flexible, gel-like material.

In the construction of O3FA biomaterials, native fish oil in triglyceride form is often utilized as the base

oil due to its unique fatty acid composition. Fish oil is unique among naturally occurring oils since it contains a high degree of monounsaturated and long chain polyunsaturated fatty acids (LC-PUFAs) in its original state prior to the oxidative crosslinking processes. The major LC-PUFAs in fish oil are the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic (DHA). The final biomaterial contains ester bonds from the original triglyceride head groups as well as ester bonds formed during the curing process (Fig. 5b). Enriched forms of fish oil in high EPA and DHA content can be blended with native fish oil in triglyceride form to alter material hydrolytic and drug delivery properties.

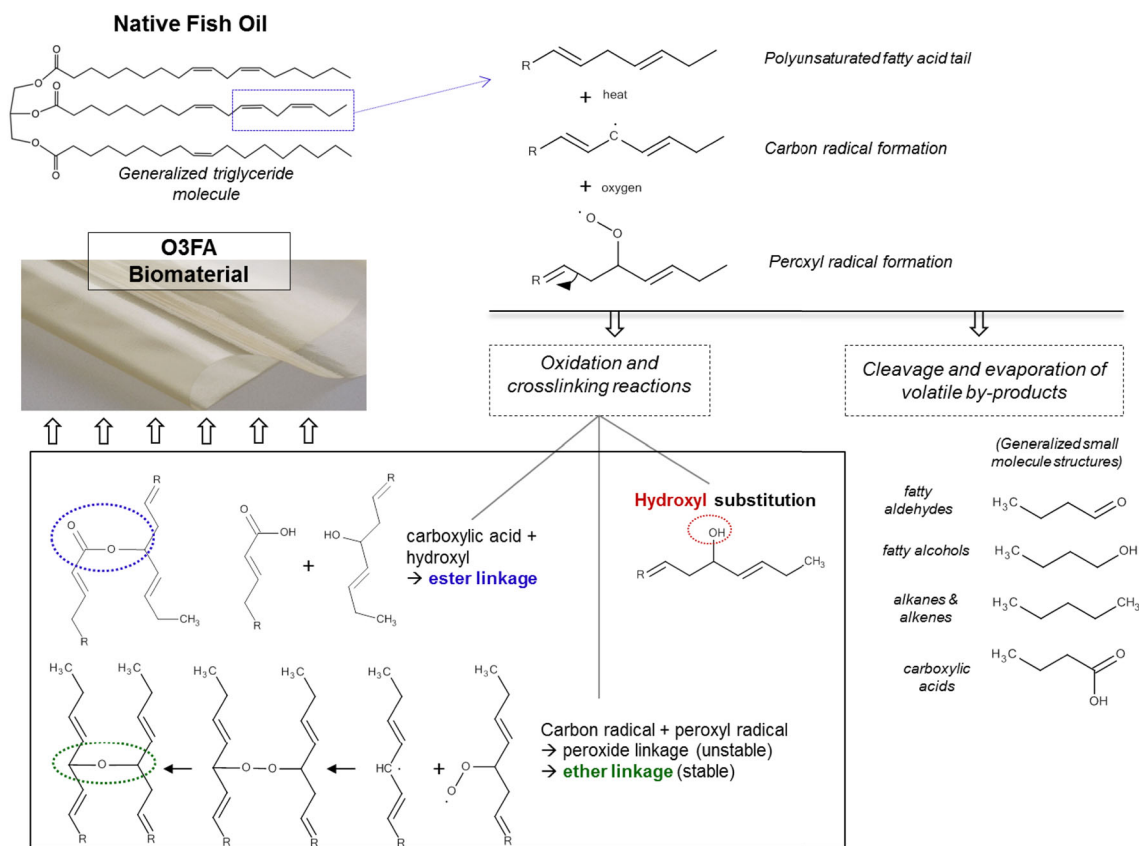


FIGURE 6. Schematic of the chemical reaction mechanisms that occur during conversion of oil starting material into the O3FA biomaterial.

Optical and Chemical Characterization Data Reveals Step-Wise Degradation of O3FA Biomaterials

In vitro and *in vivo* data provided mechanistic insight into the degradation behavior of the O3FA biomaterial. The formation and hydrolysis of esters is an equilibrium reaction (Fig. 7b) and for the equilibrium of this reaction to be shifted towards the hydrolysis of esters, sufficient amounts of water and the acidic and alcoholic hydrolysis products must be removed from the system. Evaluation of the *in vitro* and *in vivo* IVIS data for the O3FA biomaterial revealed differences in the erosion profiles (Figs. 1c vs. 2a). *In vitro*, removal of the hydrolysis products was facilitated since the O3FA biomaterials were in excess amounts of aqueous solution. Resolution of the erosion degradation rate as a function of thickness was apparent and hence could be correlated with mass (Fig. 1b). *In vivo* (Fig. 2a) it appeared that the relative rates of side chain ester hydrolysis were similar between the different thicknesses of films, however, the absolute fluorescence values differed (Fig. 2b). There was a greater resolution of film degradation observed using the GC myristic acid assay. It is theorized that this result indicates that although fluorescence can be utilized for

material tracking studies *in vivo*, a greater difference in film thicknesses between the samples to improve fluorophore signal for NBD-stearate labeled biomaterials would be required to identify different degradation rates similar to *in vitro* studies.

In the O3FA biomaterial, the amount of triglyceride esters is greater than the amount of side-chain or crosslinked esters that can form in the material (Fig. 5b). IVIS showed that the side-chain esters of the material hydrolyze first and more rapidly in the O3FA biomaterial, followed by the hydrolysis of the triglyceride esters as monitored by GC myristic acid assay. FTIR (Fig. 2d) and gravimetric measurements of dried explants revealed that the implants absorb water and proteins penetrate into the material. These studies reveal that the mechanism for the absorption of the O3FA biomaterials occurs by first absorbing water, followed by hydrolytic degradation of the side-chain ester crosslinks, degradation of the triglyceride head group esters, and finally by material absorption. This knowledge allows for the design of custom formulations where fatty acid chain crosslinking density and the ratio of ethyl ester/triglyceride esters present in the

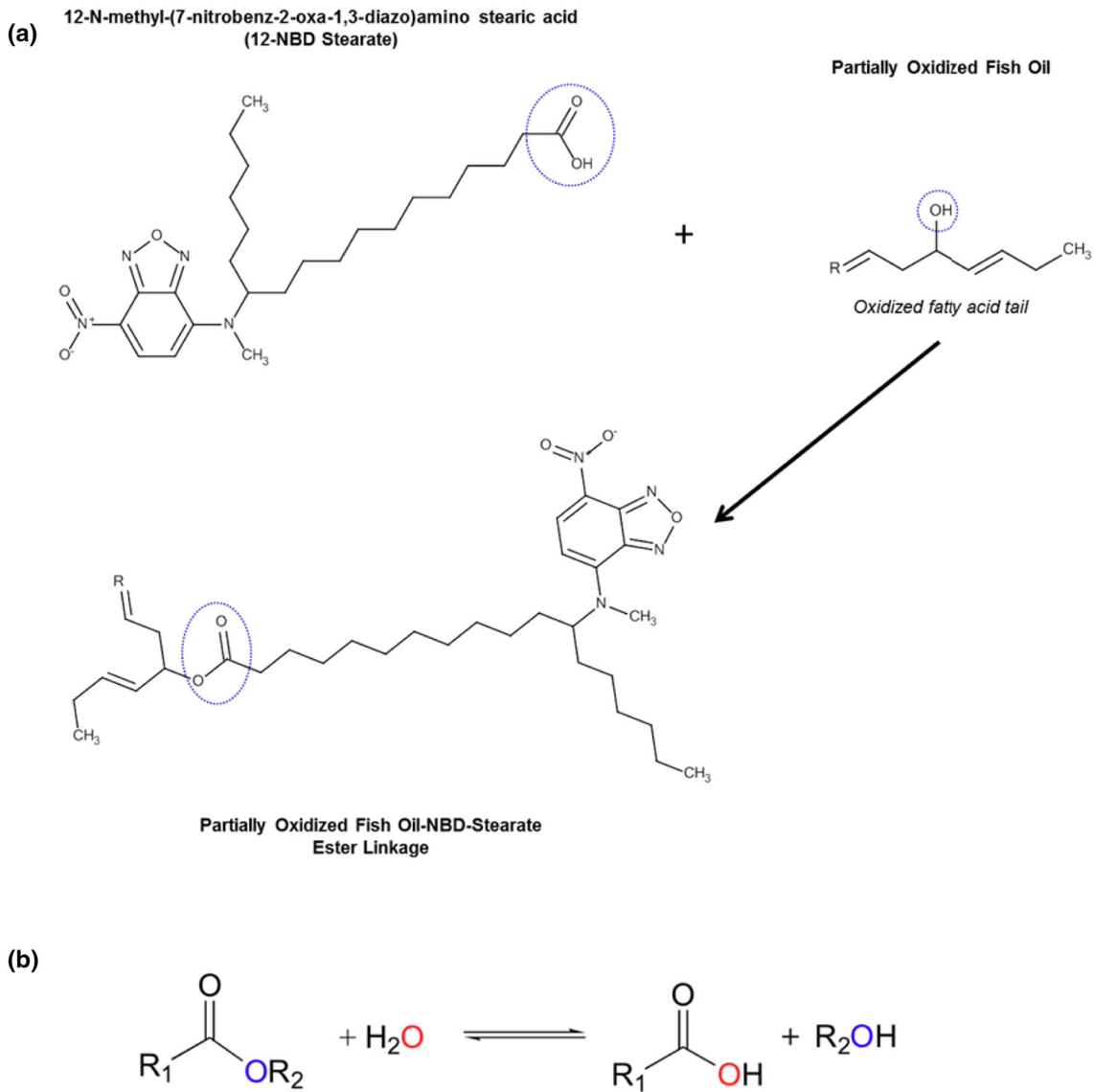


FIGURE 7. (a) Chemical mechanism for the binding of NBD-stearate with the partially thermally treated oil. (b) Chemical mechanism illustrating ester hydrolysis equilibrium.

material can be modulated in order to alter material degradation and drug delivery properties.

O3FA Biomaterial Biocompatibility and Ability to Reduce Adhesion Formation

The process used to manufacture biomaterials for medical device applications is important since the biocompatibility and toxicity of medical device materials is based on not only the chemical and physical properties of the underlying principal biomaterial, but also the extractable chemical residues that are formed during the process of material construction. The advantage in using oil and oxidative crosslinking chemistry is the minimization of manufacturing pro-

cess chemical residues and thus abrogation of their potentially toxic effects. *In vivo* evaluation of the O3FA biomaterial coated onto a bare polypropylene mesh currently used in soft tissue hernia repair applications revealed that the O3FA biomaterial coating successfully reduced adhesion area and severity. It also showed that it allowed for tissue incorporation into the mesh and led to a low inflammatory response making it attractive for use as an implanted material.

O3FA Biomaterials Mediates Drug Release

Mechanistic understanding of the process by which O3FA biomaterials hydrolytically erode *in vivo* enabled us to create oil compositions that varied by ester

content (i.e., ethyl ester or triglyceride ester) and/or EPA:DHA fatty acid content. Initial oil fatty acid composition can be conveniently altered to impart crosslinked material with a wide range of degradation kinetics. The alternation of the amount of EPA and DHA and ester form enabled significant control over drug release kinetics as well as a pronounced effect in material hydrolysis kinetics. In comparison to the fish oil control possessing an intermediate EPA/DHA concentration, there was a rapid release of drug from the coating with the higher EPA/DHA ethyl ester blend and a slow release of drug from the coating made from flaxseed oil that did not contain EPA and DHA. This demonstrated that these materials can be used for local drug delivery applications that include loading an antimicrobial or antibiofilm agent onto the surface of an implanted device.

CONCLUSIONS

Omega-3 fatty acid derived biomaterials present a promising new class of materials that can be utilized in medical device applications. Combination of multiple imaging modalities and characterization techniques enable following the graded degradation of this platform material. Understanding the degradation mechanism is crucial in controlling material properties and function. This understanding is likewise crucial in controlling embedded drug release kinetics. O3FA biomaterials, while maintaining biocompatibility, present facile tunability that allow ready modification of the physical and chemical properties. Modification of these properties in turn may alter the kinetics of *in vivo* material hydrolysis and absorption. The differences in fatty acid composition and ester form not only alters the kinetics of hydrolysis and absorption, but also can be leveraged to control drug release in local drug delivery applications.

ACKNOWLEDGMENTS

The authors would like to thank Irina Kozlova and Anthony Horner for technical support during these studies. ERE was funded in part by grants from the National Institutes of Health (R01 GM 49039).

DISCLOSURES

This work was performed using a gifted grant provided by Atrium Medical Corporation.

REFERENCES

- Artzi, N., N. Oliva, C. Puron, S. Shitreet, S. Artzi, A. Bon Ramos, *et al.* In vivo and in vitro tracking of erosion in biodegradable materials using non-invasive fluorescence imaging. *Nat. Mater.* 10:704–709, 2011.
- Aslam, S., and R. O. Darouiche. Role of antibiofilm-antimicrobial agents in control of device-related infections. *Int. J. Artif. Organs* 34:752–758, 2011.
- de Castro Bras, L. E., S. Shurey, and P. D. Sibbons. Evaluation of crosslinked and non-crosslinked biologic prostheses for abdominal hernia repair. *Hernia* 16:77–89, 2012.
- Deeken, Corey R., Keith M. Faucher, and Brent D. Matthews. A review of the composition, characteristics and effectiveness of barrier mesh prostheses utilized for laparoscopic ventral hernia repair. *Surg. Endosc.* 26:566–575, 2012.
- diZerega, G. *Peritoneal Surgery*, Vol. 1. New York: Springer, 1999.
- Doctor, H. G. Evaluation of various prosthetic materials and newer meshes for hernia repairs. *J. Minim. Access. Surg.* 2:110–116, 2006.
- Gunstone, Frank D. *Fatty Acid and Lipid Chemistry*. Gaithersburg: Aspen Publishers, Inc., 1999.
- Hartveit, F., and S. Thunold. Peritoneal fluid volume and the oestrus cycle in mice. *Nature* 504:1123–1125, 1966.
- Hirschelmann, A., G. Tchatchian, M. Wallwiener, A. Hackethal, and R. L. D. Wilde. A review of the problematic adhesion prophylaxis in gynaecological surgery. *Arch. Gynecol. Obstet.* 285:1089–1097, 2012.
- ISO. ISO 10993-18:2005, Chemical Characterization of Materials. In: Standardization IOF, editor, 2005.
- Jordan, S. W., K. M. Faucher, J. M. Caves, R. P. Apkarian, S. S. Rele, X.-L. Sun, *et al.* Fabrication of a phospholipid membrane-mimetic film on the luminal surface of an ePTFE vascular graft. *Biomaterials* 27:3473–3481, 2006.
- Lastowka, A., G. J. Maffia, and E. M. Brown. A comparison of chemical, physical and enzymatic crosslinking of bovine type I collagen fibrils. *JALCA* 100:196–202, 2005.
- Nawar, W. W. Thermal degradation of lipids. A review. *J. Agric. Food Chem.* 17:18–21, 1969.
- Ratner, B. D., A. S. Hoffman, F. J. Schoen, and J. E. Lemons. *Biomaterials Medicine an Introduction to Materials in Medicine* (3rd ed.). Boston: Elsevier, 2013.
- Storch, M. L., S. J. Rothenburger, and G. Jacinto. Experimental efficacy study of coated VICRYL plus antibacterial suture in guinea pigs challenged with *Staphylococcus aureus*. *Surg. Infect. (Larchmt)* 5:281–288, 2004.
- Turaev, A. S. Dependence of the biodegradability of carboxymethylcellulose on its supermolecular structure and molecular parameters. *Chem. Nat. Compd.* 31:254–259, 1995.