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Biodegradable lysine-derived polyurethane scaffolds promote healing in a porcine full-thickness excisional wound model

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Abstract

Lysine-derived polyurethane scaffolds (LTI-PUR) support cutaneous wound healing in loose-skinned small animal models. Due to the physiological and anatomical similarities of human and pig skin we investigated the capacity of LTI-PUR scaffolds to support wound healing in a porcine excisional wound model. Modifications to scaffold design included the addition of carboxymethylcellulose (CMC) as a porogen to increase interconnectivity and an additional plasma treatment (Plasma) to decrease surface hydrophobicity. All LTI-PUR scaffold and formulations supported cellular infiltration and were biodegradable. At 15 days, CMC and Plasma scaffolds simulated increased macrophages more so than LTI PUR or no treatment. This response was consistent with macrophage-mediated oxidative degradation of the lysine component of the scaffolds. Cell proliferation was similar in control and scaffold treated wounds at 8 and 15 days. Neither apoptosis nor blood vessel area density showed significant differences in the presence of any of the scaffold variations compared to untreated wounds, providing further evidence that these synthetic biomaterials had no adverse effects on those pivotal wound healing processes. During the critical phase of granulation tissue formation in full thickness porcine excisional wounds, LTI-PUR scaffolds supported tissue infiltration, while undergoing biodegradation. Modifications to scaffold fabrication modify the reparative process. This study emphasizes the biocompatibility and

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favorable cellular responses of PUR scaffolding formulations in a clinically relevant animal model.

Keywords

scaffold; Lysine triisocyanate; LTI polyurethane; PUR; wound repair; pig model

1. Introduction

Reports estimate that 35 million cases of significant skin loss occur each year in the United States, of which 7 million become infected and chronic with prolonged times to wound closure [1]. Best estimates suggest that over 2 million Americans currently suffer from chronic ulcers, requiring treatment costs of approximately \$8 billion per year [1]. These cutaneous defects create a need for cost-effective wound care products that actually restore skin function. Natural and biological-based tissue-engineered scaffolds that are currently available, such as Alloderm™, Integra™, Alloskin™ and Oasis™, are thin sheets that provide a temporary wound covering but are not ideal for filling deep tissue defects [2, 3]. Hydrogels have been used to deliver drugs and other biologics to wound sites; however, their small pore size and low porosity can result in delayed cellular infiltration and vascularization, and their low strength and stiffness provide limited mechanical support for tissue infiltration and remodeling [4, 5], hence not reflecting the robust viscoelastic properties that are needed as a protective covering for underlying muscles, nerves and tendons.

Polyurethane (PUR) is a biocompatible synthetic polymer that has been used in medical devices and tissue engineering applications [6]. Lysine-derived PUR scaffolds (LTI-PUR) have been shown to be biocompatible and to degrade into nontoxic decomposition products via hydrolysis of the lysine cross link [7, 8]. Furthermore, PUR scaffolds have potential for injectability and suitability as a delivery platform [9–15]. We have previously reported that injectable and implantable LTI-PUR scaffolds support cellular infiltration and extracellular matrix synthesis in rat excisional and subcutaneous wounds [14–16]. However, rat skin differs from human skin both physiologically and anatomically. Both the dermis and epidermis are thinner in rats than in humans, the vascularity is much less in rats, and loose-skinned rats heal primarily through contraction rather than production of robust granulation tissue and epithelialization [17]. The porcine wound model was chosen for its clinical relevance since pig and human skin have similar density and distribution of blood vessels, sweat glands, and hair follicles [17].

The goal of the present study was to examine early cutaneous wound healing processes in granulation tissue in the presence of a biocompatible void filler and to examine the positive or negative impact of two chemical modifications. We implanted single-layer PUR scaffolds that were synthesized from lysine triisocyanate (LTI) and a polyester triol and tested for their potential to facilitate dermal wound healing in a full thickness porcine excisional wound model, without the use of protective membranes. As a variation, carboxymethylcellulose was added as a porogen to increase scaffold permeability and

interconnectivity. Lastly plasma treatment was applied in the presence of carboxymethylcellulose (CME) to decrease the hydrophobicity of the scaffold surface. Evaluation of the degradation properties of the scaffolds and their ability to support cellular infiltration and blood vessel formation showed favorable biomaterial performance within this *in vivo* model.

2. Methods

2.1 Materials

Glycolide and D,L-lactide were obtained from Polysciences (Warrington, PA). Glycerol and the sodium salt of CMC (90 kDa) were purchased from Acros Organics (Morris Plains, NJ). TEGOAMIN33, a tertiary amine catalyst composed of 33 wt % triethylene diamine (TEDA) in dipropylene glycol, was received from Goldschmidt (Hopewell, VA). LTI was obtained from Kyowa Hakko USA (New York), and stannous octoate catalyst was purchased from Nusil technology (Overland Park, KS). All other reagents were obtained from Sigma–Aldrich (St. Louis, MO). Glycerol was dried at 10 mm Hg for 3 h at 80°C, and ϵ -caprolactone was dried over anhydrous magnesium sulfate prior to use. All other materials were used as received.

2.2 PUR Scaffold Synthesis

A polyester triol (900 Da) with a backbone comprising 60% caprolactone, 30% glycolide, and 10% lactide was synthesized by reacting a glycerol starter, cyclic ester monomers (ϵ -caprolactone, glycolide, and D,L-lactide), and stannous octoate catalyst under dry argon for 48 h at 140°C. The resulting polyester triol was vacuum-dried at 80°C for 24 h.

LTI polyurethane scaffolds (LTI-PUR) were synthesized by reactive liquid molding of the crosslinker with a hardener component comprising the polyester triol, 1.5 parts per hundred parts polyol (pphp) water, 1.5 pphp TEGOAMIN33 catalyst, and 4.0 pphp calcium stearate pore opener. LTI was added to the hardener and mixed for 30 sec in a Hauschild DAC 150 FVZ-K SpeedMixer™ (FlackTek, Inc., Landrum, SC). The resulting mixture then rose freely for 10–20 min and cured. The targeted index (the ratio of NCO to OH equivalents times 100) was 115.

For synthesis of lysine triisocyanate PUR + carboxymethylcellulose scaffolds (CMC), carboxymethylcellulose (15 wt%) was mixed with the hardener component for 30 sec prior to addition of LTI. After curing, the CMC was leached by incubating for three days in water. For preparation of PUR + CMC + plasma scaffolds (Plasma), leached CMC scaffolds were exposed to oxygen plasma for 60 sec using a Harrick Plasma PDC-001 Plasma Cleaner (Ithaca, NY).

2.3 Scaffold Physical and Mechanical Properties

Scaffold densities and porosities were determined from mass and volume measurements of triplicate cylindrical foam cores. The pore size distribution was assessed by scanning electron microscopy (Hitachi S-4200 SEM, Finchampstead, UK) after gold sputter coating

with a Cressington Sputter Coater (Vanderbilt Institute for Nanoscale Science and Engineering).

Mechanical testing was performed using a TA Instruments Q800 Dynamic Mechanical Analyzer (DMA) in compression mode (New Castle, DE). Samples were soaked in water for three days prior to mechanical testing. Stress–strain curves were generated by compressing wet cylindrical 12 mm × 8 mm samples at 37°C at a rate of 10% strain per min until they reached 50% strain. The Young's modulus was determined from the slope of the initial linear region of each stress–strain curve.

Air permeability of PUR and PUR + CMC scaffolds was determined using the constant pressure gradient method. The air flow rate (Q) necessary to maintain a pressure gradient (ΔP) of 0.12 kPa was measured, and the permeability (k) was calculated by applying Darcy's law;

$$Q = \frac{kA}{\mu} \frac{\Delta P}{L} \quad (1)$$

where L is the scaffold thickness, A is the scaffold cross-sectional area, and μ is the dynamic viscosity of air at room temperature.

2.4 Contact Angle Measurements

PUR films were synthesized for contact angle measurement since the porous structure of the scaffolds precludes contact angle measurements on the scaffolds. PUR films were synthesized by mixing polyester triol, 10 pphp TEGOAMIN33 catalyst, and LTI. The targeted index was 115. The static contact angle of a drop of water on the resulting films was measured using a Rame-Hart goniometer (Succasunna, NJ).

2.5 Porcine Excisional Wound Study

All surgical procedures were reviewed and approved by the local Institutional Animal Care and Use Committee. NIH Guide for the Care and Use of Laboratory Animals (8th Edition, 2011) were observed. The capacity of the scaffolds to facilitate healing in full-thickness cutaneous defects was evaluated in an excisional wound model (6.25 cm² square wounds) in female Yorkshire pigs (50 lbs). Treatment groups included untreated wounds (negative control, $n = 4$) and PUR, PUR + CMC, and PUR + CMC + Plasma scaffolds ($n = 6$). To mimic the chronic wound setting, full thickness cutaneous wounds that extended into the subcutaneous adipose fascial layer but spared the deep fascia encasing back muscle were created 24 h prior to scaffold implantation. This delay allowed us to advance beyond the period of hemostasis and thereby reduce the exudative characteristics of the wound bed. Bupranex (an analgesic) and cefazolin (an antibiotic) were given at the time of surgery. For the remainder of the study, the analgesic fentanyl was applied in a 50 ug/hr transdermal patch that was replaced every three days, and 500 mg of the antibiotic cephalexin was given twice daily.

Before surgery, scaffolds were trimmed into square pieces measuring 2.5 cm x 2.5 cm x 0.3 cm. All scaffolds were sterilized using ethylene oxide. Plasma treatment was applied to PUR

+ CMC + Plasma scaffolds immediately prior to implantation. Scaffolds were loosely held in place by an X-shaped configuration of spanning sutures that extended from normal skin to normal skin. After scaffold implantation, each wound was dressed with TELFA™ non-adherent dressing (Medline, Mundelein, IL), covered with Opsite™ adhesive film (Smith & Nephew, St. Petersburg, FL) beneath tube gauze, and secured with Vetwrap™ bandaging tape (3M, St. Paul, MN). Wounds were cleaned and dressings were changed every 2–3 days. Pigs were euthanized and wounds were harvested at days 8 and 15 after scaffold implantation.

2.6 Tissue Analysis

Wounds were fixed in neutral buffered formalin for 48 h, transferred into 70% ethanol for 48 h, embedded in paraffin, and sectioned at 5 µm and stained with Gomori's trichrome. Reparative responses of tissues were examined and quantified using immunohistochemical markers and procedures that we have previously validated [18]. Actively proliferating cells were immunostained for Ki-67 antigen. After heat-mediated target retrieval in citrate buffer at pH 6.0 (DAKO, Carpinteria, CA), endogenous peroxidase activity was neutralized with 3% H₂O₂ for 40 minutes followed by blocking non-specific reactivity with a casein-based protein block (DAKO, Carpinteria, CA) for 20 minutes. Slides were incubated with rabbit anti-human Ki-67 (NovaCastra Laboratories Ltd., Newcastle, UK) diluted at 1:2,000 for 60 min. The rabbit Envision HRP System (DAKO) was used with DAB as substrate and the slides counterstained with hematoxylin. Macrophage infiltration into repairing tissues was assessed using a widely used generic porcine macrophage marker - MAC387 (AbD Serotec, Raleigh, NC)[18–20]. After antigen retrieval in 0.01 M Tris/HCL pH 10, quenching for peroxidase activity, and blocking of non-specific immunoreactivity, this antibody was used at 1:10,000 for 60 min. Fragmented DNA of apoptotic cells was visualized with the DeadEnd Colorimetric TUNEL System (Promega Corporation, Madison, WI). Tissue sections were subjected to a second fixation in 4% paraformaldehyde, rinsed, and permeabilized with proteinase K for 5 minutes. The sections then were treated with equilibration buffer (Promega) followed by biotinylated nucleotide incorporation into apoptotic cells using terminal deoxynucleotidyl transferase (TdT). Endogenous peroxidase was neutralized by applying 0.3% hydrogen peroxide to the sections. Applications of streptavidin/HRP and DAB produced apoptotic-specific visible nuclear staining. Quantitative measurements were performed using Image-Pro Plus™ scientific image analysis software (Media Cybernetics, Inc., Silver Spring, MD.). Data are expressed as the total number of proliferating cells, immunolabeled macrophages, or apoptotic cells.

Density of new capillaries in the wound bed was determined by immunostaining using antisera for Von Willebrand factor. Sections underwent antigen retrieval using 5 min of digestion with Proteinase K (DAKO, Carpinteria, CA). Following rinses and endogenous blockade with H₂O₂, sections were incubated at 25° C in rabbit polyclonal antisera for Factor VIII-related antigen (; DAKO) for 30 minutes with a dilution of antibody at 1:900. Following this incubation in primary antisera, the sections were processed through the reagents supplied by rabbit Envision + System, HRP kit (DAKO). Data are expressed as area% of endothelial-lined areas within the wound.

2.7 Statistical Analysis

One way analysis of variance (ANOVA) was used to evaluate the statistical significance of results. All p-values less than 0.05 were considered statistically significant. Data are plotted as mean $\pm$ standard error.

3. Results

3.1 PUR Scaffold Physical and Mechanical Properties

The physical and mechanical properties of PUR scaffolds were characterized *in vitro* (Table 1). PUR and PUR + CMC scaffolds had similar density, porosity, pore size, and compressive modulus. However, PUR + CMC scaffolds had a 2.5-fold higher permeability than PUR scaffolds. Consistent with this analysis, SEM images in Figure 1 show that PUR + CMC scaffolds had similar pore size but higher interconnectivity than plain PUR scaffolds.

To confirm that plasma treatment increased the hydrophilicity of the scaffold surface, the contact angle of PUR films was measured before and after plasma treatment. As shown in Figure 2, plasma treatment significantly decreased the contact angle from 66° to 46° ($p < 0.005$).

3.3 Impact on PUR Scaffold Degradation

At day 8, wounds were immature and exhibited little collagen accumulation (Fig. 3A), while day 15 wounds that were treated with scaffold showed replacement of the polymer by extracellular matrix (Fig. 3B). The progression and extent of PUR degradation, measured by fractional area, decreased significantly ($p < 0.05$) from day 8 to day 15 for all scaffold treatment groups (Fig. 3C). There were no significant differences in area% PUR among the three PUR treatment groups at both time points, suggesting that the PUR + CMC and PUR + CME + plasma treatments had an insignificant effect on the rate of PUR degradation. The gradient in trichrome green intensity between the upper (light green) and lower regions (dark green) within the scaffold confirmed that the scaffold-treated wounds had the same polarity of matrix reorganization as untreated wounds.

3.4 Impact on Cell Proliferation and Apoptosis

For all treatment groups, the number of proliferating, Ki67⁺ cells decreased significantly ($p < 0.05$) from day 8 to day 15 (Fig. 4A). This result suggests that all wounds were transitioning from the proliferative phase to the maturation phase by day 15. At day 8, untreated wounds had more than three times as many Ki67⁺ cells as wounds treated with scaffolds ($p < 0.02$), suggesting that the presence of scaffold serves as a signal that dampens the proliferative response. Subtle differences in the proliferative response were detected among the three scaffold treated groups. The PUR treatment group had significantly more Ki67⁺ cells than the PUR + CMC + Plasma treatment group, which in turn had significantly more Ki67⁺ cells than the PUR + CMC treatment group. By day 15, untreated wounds had significantly fewer Ki67⁺ cells than PUR + CMC and PUR + CMC + Plasma treatment groups, suggesting that in a conventional, acute wound, the formation of granulation tissue develops and resolves more quickly in the absence of scaffolds.

TUNEL staining to assess the effect of scaffolds on apoptosis detected differential responses to treatment (Fig. 4B). At day 8, the PUR + CMC + Plasma treatment group had significantly more TUNEL⁺ cells than the PUR and PUR + CMC groups ($p < 0.01$). The number of TUNEL⁺ cells increased significantly from day 8 to day 15 in wounds treated with PUR scaffolds ($p < 0.05$). Wounds treated with PUR + CMC + Plasma scaffolds and untreated wounds had fewer TUNEL⁺ cells at day 15 than at day 8, suggesting that these two treatment groups were moving more quickly into a resolution/remodeling phase of repair by day 15.

The ratio of proliferation (Ki67⁺ cells) to apoptosis (TUNEL⁺ cells) was quantified (Fig. 4C). The ratio of Ki67⁺ cells to TUNEL⁺ cells was calculated and adjusted assuming that Ki67 is expressed for ~15 h in proliferating cells (the length of a fibroblast cell cycle [21]) and TUNEL is expressed for ~2 h during apoptosis [22]. At day 8 (Fig. 4C), untreated wounds had a significantly higher apoptosis ratio than the PUR treatment group ($p < 0.01$), which had a significantly higher ratio than the PUR + CMC and PUR + CMC + Plasma treatment groups. By day 15, there were no significant differences in proliferation/apoptosis ratio, which was slightly greater than 1 for all treatment groups. These results suggest that all wounds were moving into the remodeling phase by day 15.

3.5 Impact on Macrophage Recruitment and Persistence

Macrophage numbers typically peak in the acute wound healing setting at around 4–5 days [23]. Thus, it was anticipated that macrophage numbers would decline in the untreated wounds between day 8 and day 15 in the absence of any scaffold material (foreign body) or inflammatory stimulus. At day 8, there were no significant differences in number of MAC387⁺ population of macrophages among all treatment groups (Fig. 5). However, the number of macrophages in wounds treated with PUR + CMC + Plasma scaffolds increased significantly ($p < 0.02$) from day 8 to day 15 while the number of macrophages decreased for the PUR and untreated groups. The increase in the number of macrophages from day 8 to day 15 in the PUR + CMC and PUR + CMC + Plasma groups reflected the response to the presence of the scaffolds that contained carboxymethylcellulose.

3.6 Impact on Blood Vessel Formation

Angiogenesis is a major component of granulation tissue progression. As shown in Figure 6, the density of factor VIII-positive blood vessels (area% occupied by blood vessels) increased significantly from day 8 to day 15 in PUR, PUR + CMC + Plasma, and untreated groups ($p < 0.05$). Stated differently, the formation of granulation tissue was robust within all treatment groups and was a prominent feature during the window of time examined in this study. No significant differences in blood vessel percentage among the treatment groups were noted at either time point. These results show that treatment with the three different types of PUR scaffolds did not alter blood vessel formation during the wound healing process.

4. Discussion

PUR scaffolds were tested for their ability to safely and effectively support and possibly modify the healing properties of full-thickness porcine excisional wounds as a prelude to validating their value in the clinical setting. Two modifications were applied to blank scaffolds to determine if the empty scaffold platform could be fine-tuned to increase the degree of cellular infiltration and attachment. CMC was included as a porogen to increase pore interconnectivity and thus facilitate cellular infiltration throughout the scaffold. An additional surface modification, a plasma treatment, was applied to decrease the hydrophobicity of the scaffold surface and potentially providing a more hospitable substrate for cell attachment. *In vitro* characterization of the scaffolds showed that addition of carboxymethylcellulose increased the permeability of the scaffolds 2.5-fold, and plasma treatment decreased the contact angle by 20°. The permeability of the scaffolds is comparable to that of rigid open-cell foams reported by Zhao et al. (10^{-12} – 10^{-10} m²) [24]. When implanted in porcine excisional wounds, PUR scaffolds remained present long enough to serve as a scaffold for cellular components to fill the pores and establish an extracellular matrix. As expected the properties of the scaffold were tuned to match the rates of the scaffolding degradation with the cellular infiltration. This short-term study in the porcine model was consistent with our previous study in a loose-skinned, rodent model showing that PUR composite scaffolds stented rat excisional wounds at early time points, thereby having the potential to limit the degree of undesirable contraction and scarring [16]. However, in large cavity wounds, cosmetic outcome is secondary to encouraging the filling of the wound void.

Previous studies have investigated the use of void-filling scaffolds in porcine wound models [25–28]. A study by Huang et al. treated full-thickness porcine wounds with a bilayer dressing comprising an internal gelatin sponge layer incorporating basic fibroblast growth factor-loaded microspheres protected by an elastomeric membrane [25]. After 21 days, wounds treated with the dressing had smaller areas, thicker epidermis, and better collagen organization than control wounds treated with Vaseline gauze. Studies by Greenwood and Dearman investigated the use of biodegradable PUR foams as a temporizing matrix prior to skin graft surgery [26, 27]. Porcine excisional wounds treated with a PUR foam sealed with a microporous PUR membrane had no signs of infection and delayed contraction compared to wounds treated with Integra after 28 days. However, wounds treated with unsealed foams exhibited significant contraction and had thick scar layers above the implants.

In untreated wounds, the number of macrophages decreased from day 8 to day 15, consistent with wounds moving into resolution in the absence of scaffold material that — while transiently present — promotes a typical foreign body response [23]. As the scaffolds underwent infiltration, degradation and fragmentation between day 8 and day 15, macrophages became significantly more abundant in wounds with CMC-containing scaffolds. We have previously reported that lysine-derived PUR scaffolds degrade by hydrolysis of the polyester component at later time points as well as oxidation of the lysine component mediated by macrophages at early time points [8]. The increase in macrophages in both CMC groups provides indirect evidence that the lysine component of the scaffolds was being degraded by macrophages as we have previously shown in the rat model. It is

possible that unmodified PUR scaffolds were slower to attract monocytes due to their lower permeability and hydrophilicity than the other scaffold groups, leading to a slower rate of oxidative degradation at these relatively early time points. The anti-Mac 387 macrophage marker does not discriminate differential signaling from M1 pro-inflammatory macrophages versus M2 anti-inflammatory macrophages and their subtypes that are pivotal in wound repair [29]. Several reports have promoted macrophage phenotype as a determinant of scaffold remodeling [30, 31]. Unlike elegant studies in rodents, there is no current agreement in the literature on definitive M1 or M2 markers in either the porcine or human models [32, 33], hence an in-depth study will require considerable future investigation.

All treatment groups had significantly fewer Ki67⁺ cells at day 15 than at day 8, an indication that all wounds were moving from the proliferative phase to the remodeling phase by day 15 [23]. At day 15, the proliferation/apoptosis ratio was slightly greater than > 1 for all treatment groups. Furthermore, the number of TUNEL⁺ cells and the blood vessel area% in untreated wounds were not significantly different from scaffold-treated groups at either time point. The re-epithelialization data are difficult to interpret. It was evident that these 3 different groups had no differential impact on the rate of resurfacing. It was also evident that the process of epithelialization was not inhibited by the scaffold and the percentage of epithelial cover continued to increase between days 8 and 15 as is normally expected. At first glance it may appear that at day 15, epithelial coverage was maximal in the untreated group. However, resurfacing was enhanced by wound contraction in the non-stented (untreated) wounds. In sum, our data suggest that PUR scaffolds do not adversely affect the wound healing process in porcine excisional wounds.

There were few significant differences in wound healing properties among PUR, PUR + CMC, and PUR + CMC + Plasma scaffolds. By day 8, all three scaffolds exhibited good tissue infiltration, which suggests that the changes in interconnectivity and hydrophilicity of the scaffolds by addition of carboxymethylcellulose or plasma treatment did not significantly improve scaffold performance. We have reported that injectable, lysine-derived PUR scaffolds reduced wound contraction in rat excisional wounds. However, porcine skin has an elastic modulus of 5.9 ± 1.5 MPa [34], which is 15 times greater than that of rat skin (0.40 ± 0.15 MPa) [16] so it was critical to examine whether the scaffold would perform with satisfaction in this different viscoelastic environment. All traditional wound healing parameters in the presence of PUR scaffolds implanted in porcine excisional wounds showed biocompatibility and supported tissue infiltration and wound healing. Future work will involve testing injectable forms of PUR scaffolds in porcine excisional wounds.

5. Conclusions

In conclusion, in this large animal pre-clinical excisional skin model, all three formulations of PUR scaffolds were biocompatible, biodegradable, and supported tissue infiltration. Consistent with previous studies showing macrophage-mediated degradation of PUR scaffolds, higher numbers of macrophages were present in scaffold-treated wounds compared to untreated wounds. Taken together with analysis of proliferating cells, apoptotic cells, and blood vessels these data suggest that PUR scaffolds are safe for use as a void filler and did not interfere with essential parameters of the wound healing process.

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Figure 1. SEM images showing pore structure of PUR scaffolds. (A) LTI scaffold. (B) CMC scaffold. LTI and CMC scaffolds exhibit similar pore sizes (300 – 500 μm). CMC scaffolds have more openings in pore walls than LTI scaffolds, resulting in higher permeability. Scale bar = 750 μm .

Figure 2.

Contact angle of LTI films before and after plasma treatment. Contact decreased from 65° to 45° after plasma treatment, providing evidence that plasma treatment increases surface hydrophilicity. Asterisk indicates significant difference ($p < 0.005$).

Figure 3.

PUR scaffold degradation. (A) Representative images of trichrome staining of PUR scaffolds at day 8 at magnifications of 2X (left) and 20X (right). Scaffold fragments are marked by the letter S. (B) Representative images of trichrome staining of PUR+CMC scaffolds at day 15 at magnifications of 2x (left) and 20x (right). Fewer scaffold fragments are present at day 15 than at day 8. (C) Percentage of wound cross-sectional area occupied by residual polymer measured from images of trichrome staining. Asterisks indicate significant difference ($p < 0.05$). All scaffold-treated wounds had significantly less scaffold

material remaining at day 15 than at day 8, providing evidence that scaffolds were undergoing biodegradation in the period between 8 and 15 days. Intergroup comparison among the 3 different types of scaffolds showed no significant impact on the degree of scaffold degradation.

Figure 4.

Analysis of cell proliferation and apoptosis. (A) Ki67 immunostaining was used to analyze the presence of proliferating cells. Data are presented as number of Ki67+ cells per high power field (hpf). Asterisks indicate significant difference ($p < 0.05$). Dagger indicates significant difference from PUR+CMC and PUR+CMC+P at day 15 ($p < 0.02$). The number of Ki67+ cells decreased significantly from day 8 to day 15 in all treatment groups. At day 8, there were significant differences among all treatment groups. At day 15, PUR+CMC and PUR+CMC+P groups had significantly more proliferating cells than untreated wounds. (B) TUNEL immunostaining was used to analyze the presence of apoptosing cells. Data are presented as number of TUNEL+ cells per hpf. Asterisks indicate significant difference ($p < 0.05$). Dagger indicates significant difference from PUR and PUR+CMC at day 8 ($p < 0.01$). The number of TUNEL+ cells increased significantly from day 8 to day 15 in PUR scaffolds and decreased significantly from day 8 to day 15 in PUR+CMC+P scaffolds. At day 8, wounds treated with Plasma scaffolds had significantly more TUNEL+ cells than wounds treated with PUR and PUR+CMC+P scaffolds. (C) Ratio of proliferation to apoptosis. Dashed line represents a ratio of one. Asterisks indicate significant difference ($p < 0.01$), and dagger denotes significant difference from PUR and untreated wounds at day 8. By day 15, there were no significant differences in proliferation/apoptosis ratio among treatment groups.

Figure 5.

Analysis of the macrophage population using the MAC387 immunomarker. MAC387 immunostaining was used to analyze the presence of macrophages. Data are presented as number of MAC387+ cells per hpf. Asterisks ($p < 0.02$) and dagger ($p < 0.005$) indicate significant difference. The number of MAC387+ cells increased significantly from day 8 to day 15 in PUR+CMC+P scaffolds. At day 15, wounds treated with PUR+CMC and PUR+CMC+P scaffolds had significantly more MAC387+ cells than untreated wounds.

Figure 6.

Blood vessel formation. Factor VIII immunostaining was used to analyze the presence of blood vessels. Data are presented as area% of blood vessels within the wound. Asterisks indicate significant difference ($p < 0.05$). The blood vessel density increased significantly from day 8 to day 15 in PUR, PUR+CMC+P, and untreated wounds.

Table 1
Physical and mechanical properties of PUR scaffolds

	LTI	CMC
Density (kg m⁻³)	99.9 ± 15.3	109.8 ± 8.5
Porosity (vol %)	91.8 ± 1.3	90.9 ± 0.7
Pore size (µm)	370 ± 110	400 ± 110
Permeability (m²)	(1.9 ± 0.4) × 10 ⁻¹¹ *	(4.9 ± 0.6) × 10 ⁻¹¹ *
Compressive modulus (kPa)	18.2 ± 6.1	16.7 ± 2.9

Data are expressed as mean ± standard error.

* denotes statistical differences (p < 0.05).