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Hemostatic Nanoparticles Improve Survival Following Blunt Trauma Even after 1 Week Incubation at 50 °C

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Abstract

According to the CDC, the leading cause of death for both men and women between the ages of 5 and 44 is traumatic injury. Blood loss is the primary cause of death at acute time points post trauma. Early intervention is critical to save lives, and yet there are no treatments to stop internal bleeding that can be deployed in the field. In this work, we developed hemostatic nanoparticles that are stable at high temperatures (50 °C for 7 days) and are still effective at stopping bleeding and improving survival over the one hour time period in a rat liver injury model. These particles are exceptionally simple: PLA-based nanospheres functionalized with PEG terminated with variants of the RGD motif. This simple system can be stored at temperatures up to 50°C and maintain size, shape, and efficacy. The particles lead to a reduction in bleeding and increased acute survival with significance compared to both control particles and saline. Overall, these hemostatic nanoparticles offer an important step towards an immediate intervention in the field to stop bleeding and improve survival.

Keywords

synthetic platelet; hemostasis; bleeding; fibrinogen; artificial platelet; blunt trauma; liver injury; synthetic fibrinogen; artificial fibrinogen

Introduction

Blood loss following traumatic injury is a leading cause of death for both civilians and soldiers^{1–2}. We know that addressing bleeding during the first few minutes immediately after injury is critical: on the battlefield a soldier can bleed out in as few as 5–10 minutes after trauma, giving these casualties only ‘platinum 5 minutes’ for effective treatment to take place^{1,3}. Many civilian patients also die before reaching the hospital, and of those who do

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reach the emergency room, 35% die within 15 minutes of admission, primarily from hemorrhage⁴. Early intervention to halt bleeding is imperative to ensure that patients with severe bleeding survive to reach the hospital, where they can be stabilized and treated.

As a result, there has been great interest in developing technology to halt hemorrhage. For external bleeding, treatments such as dressings, absorbent materials, and tourniquets have been very successful⁵⁻⁶. However, there has been a lack of successful treatments for internal bleeding. Research has spanned methods ranging from blood products, cell-derived particles, drugs that interact with the clotting cascade, to synthetic materials⁷. Cell-derived products include thromboerythrocytes, thrombosomes, and synthocytes that involve modification of cell-derived materials with fibrinogen or peptides to augment clotting⁸⁻¹⁰. These approaches had some early success in vitro but there were complications in vivo including aggregation¹¹. The use of drugs such as recombinant factor VIIa (NovoSeven) and tranexamic acid looked extremely promising in early studies, but recent studies suggests their effectiveness is more limited¹²⁻²⁰. Synthetic substitutes include polymer nanoparticles and liposomes that interact with activated platelets to enhance clotting²¹⁻²³. These particles have shown promise in halting bleeding, and research into their use is ongoing. However, one of the challenges with many polymer systems involves the temperature range over which they remain effective. To be effective these treatments need to be administered within minutes of injury, but many polymer systems are not stable at extreme temperatures limiting whether they could ever be considered for use in the field. The temperature in Afghanistan can reach 50 °C²⁴.

Our lab has developed a synthetic substitute using nanoparticles having a poly(lactic-co-glycolic acid) (PLGA) core and poly(ethylene glycol) (PEG) arms that can interact with activated platelets via the glycoprotein IIb/IIIa receptor to reduce bleeding and increase survival in blunt and blast trauma^{23, 25-27}. While these particles are stable at room temperature (27 °C for over a week), they are not stable at extreme temperatures. When stored at 37 °C for one week, the hemostatic efficacy of the particles was compromised²³.

However there are nanoparticle formulations that can withstand higher temperature environments. Poly(lactic acid) (PLA) has similar properties to PLGA but has a higher glass transition temperature, can exhibit some crystallinity, and is able to form stereocomplexes by blending its two isotactic stereoisomers poly(d-lactic acid) PDLA and poly (L-lactic acid) PLLA, which may further enhance core stability²⁸⁻²⁹. In this study we investigated the properties of nanoparticles having a PLA core, and the impact of storage at extreme temperatures on the stability of PLA-PEG-GRGDS nanoparticles. We evaluated the effects of temperature by monitoring particle size both before and after exposure to high temperature, and by testing the hemostatic efficacy of heat-treated particles in a rodent blunt trauma model. In addition, we validated the mechanism of action of the hemostatic nanoparticles through the glycoprotein IIb/IIIa receptor. This study is a critical step towards the development of a translatable treatment for internal bleeding.

Materials and Methods

Materials

Poly (ethylene glycol) (HO-PEG-CM Mn 5,000) was purchased from Laysan bio. L-lactide was purchased from Polysciences Inc. D-lactide was from PURAC biomaterials. 1,3-Bis-(2,4,6-trimethylphenyl)imidazole-2-ylidene (IMes) was purchased from Sigma Aldrich. CDMFA (cell tracker green) was purchased from Life Technologies. All reagents were ACS grade and purchased from Fisher Scientific.

Methods

Polymerization—PLA and PLA-PEG block copolymer were generated via ring opening polymerization catalyzed by IMes using a procedure based on Conner et al.²⁸ For the PLA-PEG block copolymer the hydroxy group on heterobifunctional PEG was used as the initiator for the polymerization. Lactide and PEG were dissolved in dichloromethane (DCM) in an inert atmosphere (170:1 molar ratio). A solution of IMes in DCM was then added for a ½ molar equivalent to PEG. The reaction proceeded for 1 hour and was terminated by the addition of two drops of acetic acid. The polymer was precipitated in methanol and collected. PLA was polymerized by dissolving a solution of lactide in DCM and adding methanol for a 170:1 molar equivalent of lactide:methanol. A solution of IMes in DCM (1/2 molar equivalent to methanol) was then added and the reaction proceeded for 1 hour before terminating with acetic acid and precipitation in methanol.

Peptide Conjugation—GRGDS peptide (or the control substitute GRADSP) was conjugated to the PEG by dissolving the copolymer in DCM. A mixture of EDC and NHS in 10 molar excess was dissolved in dimethyl sulfoxide (DMSO) and added to the polymer solution and allowed to stir for 1 hour. The polymer was precipitated in methanol, filtered, and dried on the lyophilizer for 1 hour. The polymer was then redissolved in a DCM/DMSO solution to which a 10 molar excess of peptide was added. This solution was stirred for 24 hours, then precipitated and lyophilized.

Nanoprecipitation—A mixture of PLA and PLA-PEG-GRGDS was dissolved in THF at 20mg/ml. This solution was added dropwise to a stirring solution of PBS twice the volume of the THF. Nanoparticles formed as the water miscible solvent dissipated within the PBS. After stirring for 1 hour, poloxamer was added to the solution in a 1:1 weight ratio to the PLA/PLA-PEG-GRGDS. The nanoparticle solution was then dialyzed to remove residual solvent, snap frozen, and lyophilized.

PLGA-PLL-PEG-GRGDS nanoparticles were synthesized as previously described.²⁵ Briefly, the PLGA-PLL-PEG-GRGDS polymer was dissolved in acetonitrile at 20 mg/ml. This solution was added dropwise to a stirring solution of PBS twice the volume of the acetonitrile and the nanoparticles formed as the solvent dissipated in the PBS.

Characterization—Molecular weight of the product was determined by gel permeation chromatography (GPC) (Shimadzu). Glass transition and melting temperatures and crystallinity were determined by differential scanning calorimetry (Q100 TA instruments).

Particle size was determined by dynamic light scattering (DLS 90Plus, Brookhaven Instruments Corporation) and scanning electron microscopy (Hitachi S4500).

The presence of peptide in the polymer was confirmed by hydrolysis of the product and use of the o-phthalaldehyde reagent to detect free amines. The hydrolysis procedure followed was previously described.²³ Briefly, a 5mg aliquot of polymer was hydrolyzed for 24 hours in a hydrolysis/derivatization workstation (Eldex Laboratories, Napa, CA). The hydrolysate was neutralized with a 1 M sodium hydroxide solution. This solution was tested using an o-phthalaldehyde (OPA) kit (Anaspec, San Jose CA), which fluoresces in the presence of free amines. The fluorescence was detected using a plate reader (Molecular Devices Spectramax M3).

Mechanism of Action—Collagen I (rat tail) was suspended at 500 ug/ml. Polymers (PLA-PEG and PLA-PEG-GRGDS) were dissolved at 5 mg/ml in trifluoroethanol. One hundred uL of solution was added to each well of a 96-well plate and the solution was allowed to evaporate to coat the well. Each well was then rinsed 3X with PBS. Untreated wells were used as a negative control. Blood was drawn from male Sprague-Dawley rats and centrifuged to isolate platelet rich plasma (PRP). The platelet rich plasma was fluorescently labeled with CDMFA and diluted with bovine serum albumin (BSA) to a platelet count of 5×10^8 platelets/ml as measured by a Coulter Counter (Multisizer 3). Two dilutions were made, one containing only PRP and BSA, and the other containing a 0.04 mg/ml concentration of eptifibatide, an inhibitor of the GPIIb/IIIa receptor. These were allowed to incubate at room temperature for 10 minutes, at which point 100 ul of the platelet solution was added to each well, followed immediately by 10ul of 100 uM adenosine diphosphate (ADP). The plate was agitated for 1 minute on an orbital shaker, followed by 5 minutes of equilibration. The plasma and unaggregated platelets were extracted and the fluorescence of each well was measured in the plate reader (ex: 485 nm, em: 525 nm).

Temperature Stability Testing—Aliquots of hemostatic nanoparticles (GRGDS) and control (GRADSP) nanoparticles were stored in a dry state in an oven held at 50°C for 7 days to determine the effects of high temperature on the particles. Particle size was measured following storage to determine if aggregation had occurred.

In vivo liver injury model—Particle effectiveness was evaluated in a rodent blunt trauma injury model as previously described elsewhere, which was adapted from Ryan et al. and Holcomb et al.^{30–31} This work was approved by and undertaken according to guidelines set by Case Western Reserve University's institutional animal use and care committee. The primary outcomes recorded were blood loss and survival at 1 hour following injury.

Briefly, Sprague-Dawley rats (225–275g, Charles River) were anesthetized with an intraperitoneal injection of a mixture of ketamine/xylazine (100:10 mg/kg respectively). After 10 minutes they were shaved and placed on a heat pad. A tail vein catheter (24G $\times$ $\frac{3}{4}$ inch Exlet safety catheter) was placed prior to incision. The animal was then placed in a supine position and a midline cut was made to expose the liver. The medial lobe of the liver was marked using a hand-held cautery device in a 1.3 cm arch from the suprahepatic vena cava. The medial lobe was then resected along the marked lines and the abdomen was closed

with wound clips. Treatment was immediately administered in a 0.5cc bolus through the tail-vein catheter followed by 0.2cc saline to flush the catheter dead-volume.

Rats were allowed to bleed for 1 hour or until death, as determined by the lack of breathing or a palpable heartbeat. Rats were then injected with a lethal dose of sodium pentobarbital. The abdomen was re-opened and blood was collected using preweighed gauze. To prevent additional bleeding, the adherent clot on the liver was collected last. The resected portion of the liver was weighed and preserved in a 4% paraformaldehyde solution. In addition, the remaining liver was removed and preserved in 4% paraformaldehyde.

Statistics—Treatments included saline (n=21), control nanoparticles (n=21), and hemostatic nanoparticles (n=21). Particle treatments were suspended at 20 mg/ml in PBS. The surgeon was blinded to treatments during the experiment. Survival was analyzed in R using the Kaplan Meier Estimate with 95% confidence bounds. Blood loss was analyzed using ANOVA with Tukey post-hoc comparisons.

Results

Melting of PLGA-based hNPs versus PLA-based hNPs

In our original development of hemostatic nanoparticles (hNPs), we found that the PLGA-based particles were not effective when stored at 37 C²³. Here, we incubated the particles for 5 days at either 37 C or 50 C to look for signs of melting. As expected, the PLGA hNPs showed signs of melting at both 37 C (figure 1A) and 50 C (figure 1C). In contrast, the PLA-based particles described in depth below, did not show signs of melting at either 37 C (figure 1B) or 50 C (figure 1D). This finding motivated the in vitro and in vivo characterization.

Polymer Synthesis

Block PDLA-PEG was synthesized using an N-heterocyclic carbene catalyst as described by Conner et al. and Shin et al., with the substitution of heterobifunctional PEG (HO-PEG-CH₂COOH) as the initiator^{32–33}. Our block PDLA-PEG copolymer had an approximate molecular weight 35 kDa as verified with size exclusion chromatography (SEC) indicating that the PDLA block was approximately 30 kDa with a PDI for the copolymer of 1.44 (figure 1E). The PLLA (with a methanol initiator) had a similar Mn of around 28 kDa. This was within our desired size range. DSC data indicated that the thermal properties of the PLLA were within the range expected for PLA of that Mn. The glass transition temperature of the block copolymer was slightly lower, indicating the effect of the presence of a PEG block, an effect that has been previously observed in PLA-PEG polymers, and which is consistent with the value calculated by the Fox equation ($1/T_g = w_1/T_{g1} + w_2/T_{g2}$) of 44.5 °C, suggesting that PLLA and PEG are completely miscible^{34–35}.

Following conjugation of the peptide, OPA analysis was used to confirm that the peptide had successfully coupled to the polymer. The copolymer was hydrolyzed, and the product was reacted with OPA, a reagent that fluoresces in the presence of free amines from the conjugated peptide. OPA analysis indicated that approximately 17% of the available PEG sites had peptide conjugated.

Particle Synthesis and Characterization

Particles formed by blending these polymers could be tuned to have diameters between 100 nm and 500 nm by adjusting the ratio of PDLA-PEG and PLLA, which was confirmed by DLS and SEM (figure 1B and 1F). This variation was not linear, but appeared to reach a critical point with lower PEG ratios, possibly resulting from the reduction of PEG surface density. Because we wanted to develop a particle similar to our previous system using PLGA nanospheres, our follow up experiments used nanospheres made by blending PLLA and PDLA-PEG in a 3:1 ratio by weight. However, if a higher PEG density was desired, this system could be tuned to increase the total percent of PEG.

Dosing and Biodistribution

Initial testing of the hemostatic nanoparticles (hNPs) was performed on a small cohort of animals using a blunt trauma model previously described elsewhere.²⁵ Initial testing to determine dose indicated that 40 mg/kg was an appropriate dose (figure 2). No animals from the initial 40 mg/kg group died before the 1 hour time point. Therefore this dose was used for subsequent testing. In addition, biodistribution was performed on the animals in this group to determine where the particles traveled in the body after injection. Biodistribution was performed during this initial study because the molecule used as an indicator for the nanoparticles does not remain encapsulated at high temperatures. Biodistribution data indicated that particles were found primarily (53%) in the liver, which was the site of injury. About 70% of the nanospheres were recovered from the organs tested. Unrecovered particles were most likely located in the shed blood. Ten percent of the particles were located in the lungs, and the remaining organs contained less than 3% of the total dose.

High Temperature Testing

Following the initial dosing experiment, particles used in subsequent experiments were stored at 50°C for 1 week to determine if this resulted in any loss of particle form or function. Particles were sized using both DLS and SEM (figure 3). The hydration shell created by the PEG arms accounts for the difference in size seen between DLS and SEM. Following storage at 50 degrees, both DLS and SEM data indicated that particle size may trend toward a slightly increased size, although this change was not statistically significant (DLS results $p=0.57$, SEM $p=0.61$).

Mechanism of Action

The aggregation of platelets on surfaces coated with different polymers was measured by fluorescently tagging platelets following our previous work²³. Aggregation is significantly higher in wells treated with collagen than in untreated wells (figure 4a). Aggregation in wells containing PLA-PEG-GRGDS polymer has significantly increased aggregation compared with control wells (figure 4b) which is similar to what has been seen previously in our work²³. In addition, when eptifibatide a cyclic heptapeptide that rapidly binds to the glycoprotein IIb/IIIa receptor inhibiting it, is included, platelet aggregation is decreased to levels that are not significantly different from controls (figure 4b). Eptifibatide competitively binds with the glycoprotein IIb/IIIa receptor indicating that the glycoprotein IIb/IIIa receptor is critical for the mechanism of binding of activated platelets to hemostatic nanoparticles.

Survival at 1 hour

Because the particles did not appear to change significantly following high-temperature storage, they were resuspended at the previously determined dose of 40 mg/kg for testing in the rat blunt trauma model. Of the 69 animals injured, an injury consistent with our model was produced in 63 animals, 21 in each of the three treatment groups. The results from the rat liver injury model (figure 5) demonstrated that the particles were effective in reducing bleeding and increasing acute survival. Our previous work using PLGA hemostatic nanoparticles in this model had shown increased survival by 50% at the one-hour time point, which is similar to the effect seen here.²⁵

Survival in the treatment group (hNPs) was significantly increased compared with control nanoparticles (Kaplan Meier Estimate with 95% confidence bounds, $p = 0.000271$). Survival was also significant compared to saline ($p = 0.0347$). There were no significant differences between the three groups in resected liver mass, indicating that the injury was carried out consistently.

Blood Loss

We know from our previous work^{25–26} that the PLGA core nanoparticles effectively reduced bleeding. Although the method of determining blood loss by measuring the weight change in gauze used to absorb the blood shed into the body cavity lacks fine resolution, there appears to be a trend of slightly reduced blood loss in animals that received the hemostatic nanoparticle (hNP) treatment (figure 5B).

It is striking that small changes in blood loss can have such a substantial effect on survival. What can be seen here in the rodent model is replicated in humans. There is a critical amount of blood a person can lose and survive. Past this point, lethality is extremely likely.

Comparison of Treatments

In order to determine if the heat treatment caused any change in the effectiveness of the particles, we carried out a second liver injury study comparing heat treated particles, particles that had not been heat treated, and the PLGA-PLL-PEG-GRGDS particle formulation that these particles are designed to replace. First, we determined that the injury model was reproducible among the groups tested with the same amount of liver, within the tolerances of the model, being resected (Figure 6A).

Blood Loss between PLGA hNPs and PLA hNPs

There was no significant difference in blood loss between the three groups, the PLA hNPs stored cold and resuspended, the heat-treated PLA hNPs, and the PLGA hNPs (Figure 6B).

Survival at 1 hour between PLGA hNPs and PLA hNPs

All PLA particles for this study were resuspended at 40 mg/kg. The PLGA core nanoparticles were resuspended at 0.5 mg/kg, the dose used for previous studies. Of the 30 animals tested, an injury consistent with our model was produced in 26 animals: 9 in each PLA hNP group and 8 in the PLGA hNP group. The results of this study (figure 6C) demonstrated that there was no significant difference in survival between the three groups

(PLA hNP after storage at 50 °C vs. before treatment OR = 1.00, CI = 0.108–9.229, PLA hNP after storage at 50 °C vs PLGA hNPs OR= 0.857, CI = 0.091 – 8.075). We did not test any heat treated PLGA particles since they flow at 50 °C.

Discussion

There is a very brief window of time to intervene before a patient bleeds out following trauma, and this creates a great need for a treatment that can reach a patient before they reach a medical facility. One of the requirements for a treatment to be transported to the patient is that it has to be thermally stable over significant temperatures, up to 50°C. These PLA-based hNPs improve survival following blunt trauma even after they are stored at 50°C for 7 days. This is an important step in developing therapies that can be used by first responders to reduce bleeding and improve survival.

The stability of the nanoparticles was enhanced by using PLA as the core material so that particles maintained their physical structure when stored at 50°C as demonstrated by DLS and SEM observations (figure 3). The presence of PEG on the polymer reduced the glass transition temperature according to DSC data (figure 1), however when the polymer was made into nanoparticles, the core of the particle was primarily PLA, which can remain in a solid state up to 50°C. It may be possible to reach even higher temperatures without loss of stability because of the stereocomplexes formed between PDLA and PLLA. We focused on the 50°C temperature because it has been reported as a critical temperature at which materials must be stable to be used in the extreme environments experienced by the military

Platelet aggregation data demonstrates that hNPs do act through the glycoprotein IIb/IIIa receptor, which is the receptor that binds to the peptide GRGDS. Eptifibatide inhibits the receptor. When the receptor was inhibited, platelets aggregate on PLA-PEG-GRGDS significantly less (figure 4B), at levels similar to aggregation on an untreated surface. In addition, the PLA-PEG copolymer did not have significantly greater aggregation than the untreated surface, also suggesting that the presence of the GRGDS peptide was necessary to bind platelets.

Survival data (figure 5) indicated that the particles had a significant effect on increasing survival compared to the control nanoparticles, which were similarly heat-treated. There was an apparent trend towards a reduction in lethality when compared to the saline treated animals. This trend is accordance with the effects we have seen using previous generations of hemostatic nanoparticles, which increased survival by 50% when compared with saline^{25–26}. In addition, we tested the particles in a direct comparison with PLA nanoparticles that had not been heat-treated and with our previous generation of PLGA core hNPs. We found that heat treatment had not apparent effect on the efficacy of the particles in reducing lethality. Together, these studies demonstrate that the PLA core particles are an effective treatment for hemorrhage and that heat treatment does not significantly affect their size, shape, or efficacy.

No significant difference was observed in the blood loss data between the groups of animals, although the hNP treatment group did trend towards a reduction in blood loss compared to

saline. Previous studies have had mixed results in blood loss trends using this metric^{23,25}. Although this measurement cannot detect small differences in blood loss, it is encouraging that the survival data and blood loss data indicate similar trends, suggesting that the particles are likely to be reducing bleeding which, in turn, results in reduced lethality at one hour. Small differences in bleeding can have a large impact in survival²⁵.

Biodistribution data indicated that the majority of the particles were found in the liver, which was the site of injury. This is consistent with our results using previous generations of hemostatic polymer nanoparticles^{25–26}. Approximately 10% of particles were located in the lungs, which could be a result of microemboli occurring during bleeding or from nanoparticles still in pulmonary circulation. However, this result is also consistent with previous work, and no indications of pulmonary embolism were observed in treated animals^{25–26}.

One of the many questions that remains is how long after injury these particles may be effective. We know that earlier administration of hemostatic agents leads to better outcomes. We also know that there is a critical volume of blood loss than can be tolerated before the body decompensates dramatically. The time after the initial injury over which the particles may be effective will likely be a function of the extent of injury, the rate of blood loss, and whether other measures are being used to stabilize the patient.

Conclusion

Blood loss is a leading cause of death in both civilian and combat settings.^{5,6} Currently there are no treatments that effectively deal with internal hemorrhage, which must be reduced within minutes of injury to improve patient survival. In addition, to be practical for use by a first responder, a drug cannot require excessive special handling such as refrigeration to ensure that it remains safe and effective until use. We have developed a hemostatic nanoparticle with a PLA core that remains as effective a hemostatic agent as our previous generation of PLGA hNPs following storage at 50°C. This finding is critical towards translation of this technology into clinical use. Further work is needed to further assess efficacy of these particles in a large animal model and to more completely define safe storage conditions.

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Figure 1.

(A–B) SEMs of hNPs held at 37°C for 5 days. (A) PLGA spheres are starting to show signs of melting. (B) PLA-based spheres show no signs of melting. (C–D) hNPs held at 50°C for 5 days. (C) PLGA spheres are totally melted. (D) PLA-based spheres show no signs of melting. (E) Size and temperature characteristics of PLLA and PDLA-PEG polymer products. (F) Tailoring size of PLLA/PDLA-PEG nanoparticles through PLLA/PDLA-PEG blends as measured by DLS. The PDI for each value varied between 0.05 and 0.24 suggesting that the particles had reasonably low polydispersity of size.

Figure 2.

(A) Pilot study of 1 hour survival of several doses of PLA hNPs. 40 mg/kg was optimal in this dosing study and used for the remainder of the work presented here. (B) Biodistribution of nanoparticles in organs for the optimal dose (40 mg/kg)

Figure 3.

(A) DLS and SEM sizing of PLA-PEG nanospheres before and after exposure to a 50°C environment for 7 days. The PDI for the spheres before treatment was 0.124 and after heat treatment was 0.178. (B) SEM micrograph of PLA hNPs as made, prior to exposure to 50°C. (C) PLA hNPs following exposure to 50 °C temperature treatment remain separate and spherical. Both DLS and SEM show no significant changes in particle size, shape, or aggregation. The differences in both DLS and SEM are not significant.

Figure 4.

(A) Platelet aggregation measurement. Platelets aggregate significantly more ($p < 0.05$) in wells treated with collagen. (B) Platelets aggregate significantly more ($p < 0.05$) in PLA-PEG-GRGDS compared with control wells. The addition of eptifibatide, a GIIb/IIIa inhibitor, reduces the aggregation down to the control level confirming the role of the receptor binding to the RGD moiety as a mechanism for aggregation of activated platelets to the material.

Figure 5.

(A) Resected liver as a percentage of body weight was not significant ($p=0.3141$). (B) Blood loss is not significant between groups, although it appears that hNP treatment trends towards a reduction in blood loss ($p=0.7273$). (C) Survival curve following liver trauma. (D) Survival of animals at one hour following liver trauma. The hemostatic nanoparticle (hNP) treatment (PLA-PEG-GRGDS nanoparticles) are significant compared with control nanoparticles (PLA-PEG-GRADSP) and saline (Kaplan Meier Estimate with 95% confidence bounds; Control vs. Saline, $p=0.0436$; Control vs. hNP, $p=0.000271$; hNPs vs. Saline, $p=0.0347$)

Figure 6.

Liver injury results for comparison of three hemostatic nanoparticle formulations (A) Average resected liver demonstrating consistent injury. (B) Blood loss measured from animals in this study. (C) Survival as a function of particles. The PLA particles stored both cold at hot (50°C) showed the same blood loss and survival as the previously successful PLGA hemostatic nanoparticles.