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Host-biomaterial interactions in zebrafish

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Host-biomaterial interactions in zebrafish

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ABSTRACT

Host-biomaterial interactions are critical determinants of the success or failure of an implant. However, detailed understanding of this process is limited due to a lack of dynamic tools for *in vivo* analyses. Here we characterize host-biomaterial interactions in zebrafish (*Danio rerio*), which are optically translucent and genetically tractable. Histological and immunohistochemical analyses following polypropylene suture implantation into adult zebrafish showed prolonged elevation of immune cell recruitment and collagen deposition, resembling a foreign body response. Live *in vivo* analysis showed that adsorption of the immunomodulatory cytokine interleukin-10 to a polystyrene microparticle, microinjected into transgenic larval zebrafish, inhibited neutrophil recruitment after 24 hours compared to control microparticles, with no change in macrophage recruitment. This study illustrates that zebrafish are useful to investigate host-biomaterial interactions and have potential for high-throughput analysis of novel immunomodulatory biomaterials.

KEYWORDS

zebrafish, biomaterial, inflammation, immunomodulatory, interleukin-10

A Letter to the Editor

Immune cell-biomaterial interactions are critical for the success or failure of implanted biomaterials. For example, immune cells may isolate the biomaterial in a scar-like, avascular fibrous capsule in a process known as the foreign body response.¹ On the other hand, positive interactions with immune cells may lead to biomaterial vascularization and integration with the surrounding tissue.^{2, 3} Several studies have explored the potential for developing biomaterials that will modulate the immune

cell response by altering biomaterial surface topography⁴ and chemistry,⁵ incorporation of bioactive molecules,^{6, 7} or combinatorial approaches,⁸ for enhanced healing, integration, and vascularization. However, a major challenge in evaluating new technologies is our limited understanding of the complex mechanisms underpinning immune cell-biomaterial interactions, which is due, in large part, to limited *in vivo* and *in vitro* models with which to investigate these interactions in real time.⁹

Currently, biomaterials are evaluated for biocompatibility via *in vitro* cytotoxicity analyses, which are oversimplified, and implantation in rodent models, which are costly, time-consuming, and largely rely on fixed histological endpoints.¹⁰ More advanced but non-standardized methods have been explored to study novel biomaterials *in vivo* in real time, such as the dorsal skinfold window chamber¹¹ and injection of chemiluminescent drugs that fluoresce in the presence of reactive oxygen species.¹² Recently, the combination of a dorsal skinfold window chamber and infrared-excited non-linear multiphoton microscopy was used to visualize the longitudinal host-biomaterial response to 3D-electrospun scaffolds in mice, including multi-nucleated foreign body giant cell formation derived from macrophages and second harmonics generation to reveal collagen deposition and structure.^{13, 14} While these studies and techniques have provided a window into the *in vivo* biomaterial environment, more tools are needed for directly interrogating the behavior of immune cells themselves, especially against the backdrop of disease. One model organism that allows *in vivo* imaging and relatively easy genetic manipulation for the study of human disease, but in which immune cell-biomaterial interactions have not been thoroughly characterized, is the zebrafish (*Danio rerio*). Zebrafish provide distinct advantages over other *in vivo* models because there is a wide library of existing transgenic fluorescent lines and they are inexpensive, optically translucent, genetically modifiable, reproduce rapidly, and allow whole-animal live imaging analysis.¹⁵ Previous studies have successfully utilized zebrafish for high-throughput visualization of skeletal structures and mutations,^{16, 17} along with drug discovery screening, including kinetic exploration of the mechanisms of action,¹⁵ which is particularly challenging in rodents.¹⁸ Transgenic zebrafish have been used extensively as model organisms to study human diseases, including cancer¹⁹ and

gastrointestinal disease,²⁰ which are becoming increasingly important in the design and testing of new biomaterial therapies.²¹

Adult zebrafish also have been shown to be an excellent model of acute skin wound healing, wherein leukocyte infiltration and collagen deposition peak between one and six days post wounding (dpw), and then progressively resolve by 10 dpw so that wounds become indistinguishable from unwounded controls.²² One study utilized the implantation of a small ceramic microparticle to induce the recruitment of melanocytes, via the prolonged actions of host immune cells.²³ Additionally, several studies have utilized zebrafish larvae to explore nanoparticle toxicity *in vivo*.²⁴⁻²⁶ Together, these studies suggest that zebrafish may be a useful model for studying the inflammatory response to implanted biomaterials. Zebrafish are uniquely positioned with strong potential for genetic and dynamic analyses of immunomodulatory biomaterials in normal and diseased models in real-time. Therefore, the goals of this study were to first characterize host-biomaterial interactions in zebrafish, and second, to observe the effects of an immunomodulatory biomaterial in real-time.

In this study, as a proof of concept, monofilament polypropylene sutures, a biomaterial known to elicit a well-characterized classic inflammatory response *in vivo* across many species,²⁷ were implanted into adult wild-type zebrafish. These fish were later sacrificed at time points ranging from one hour to 21 days post-implantation (dpi) for histological and immunohistochemical analyses. Next, varying sizes of polystyrene microparticles, a non-degradable and hydrophobic synthetic biomaterial similar to polypropylene,²⁸ were microinjected into the skin and muscle in the dorsal lateral flank adjacent to the cloaca in four day post-fertilization (dpf) transgenic larval zebrafish, which are more translucent compared to adult zebrafish and thus facilitate live image analysis. Finally, to observe the effects of an immunomodulatory biomaterial in real time, transgenic larval zebrafish were microinjected with a 25µm polystyrene microsphere with and without adsorbed interleukin-10 (IL10), an immune-cell modulating cytokine. Using live confocal imaging analysis, the numbers of neutrophils and macrophages drawn to the microparticle were quantified for up to seven days after implantation and compared to wounded and unwounded controls.

To ensure the suture would remain in place in the adult zebrafish for the duration of the study, it was looped through the entire tail, keeping the scales intact to aid in holding the suture in place, and fish were single-housed (**Figure 1A & B**). Adult transgenic zebrafish implanted with a polypropylene suture were imaged using multiphoton microscopy, allowing the visualization of immune cell behavior and collagen structure around the suture, via fluorescently labeled neutrophils in green, macrophages in red, and second harmonics generation for collagen visualization (**Figure 1C-F**). To enable traditional host-biomaterial interaction characterization, tissue samples ($n \geq 3$ zebrafish per group per time point) of zebrafish implanted with the suture, as well as control wounded zebrafish in which a suture was implanted and then immediately removed, were embedded in paraffin, transversely sectioned, stained with Masson's trichrome for collagen, and quantitatively analyzed for collagen capsule thickness using Fiji software.²⁹ The results showed a steadily increasing collagen capsule thickness surrounding the suture over time from 1hpi to 21dpi (**Figure 1G-K, Q**). Interestingly, 7dpi is about when collagen deposition peaks following the response to normal cutaneous tissue damage in zebrafish, but in an acute wound scenario collagen levels return to baseline by 10dpi.²² However, with a suture, collagen deposition continued to significantly increase beyond the 7dpi time point, with 10dpi and 21dpi exhibiting significantly greater collagen area compared to all earlier time points (**Figure 1Q**). In contrast, the histological response to control wounds was impossible to identify beyond a few hours after suture removal when the wounds still exhibited early bleeding and redness, as seen in the 1hpi image (**Figure S1A**). By 3 dpi (**Figure S1B**), the wounded control appeared no different from unwounded tissue, while fish with implanted sutures continued to have elevated redness and hyperpigmentation surrounding the suture until 21dpi (**Figure S1F-J**), consistent with histological observations shown in **Figure 1**. These results, in combination with previously published characterization of the zebrafish response to cutaneous wounding,²² suggests that the observed response was a result of the implanted biomaterial as opposed to the wound itself. However, developing and optimizing an appropriate tracking mechanisms for wounded control fish may be useful in future work.

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Next, we examined the total number of leukocytes surrounding the suture using immunohistochemical staining for L-plastin, which serves as a pan leukocyte marker that labels all innate immune cells^{30, 31} (**Figure 1L-P**). In zebrafish L-plastin is expressed in the earliest primitive macrophages from 24 hours post fertilization, and is maintained through adult stages.³² The presence of the suture led to a significant increase in innate immune cell numbers (from the 1hpi to 3dpi time points). The elevated immune cell counts were sustained until the end of the experiment at 21dpi (**Figure 1R**), in contrast to a normal acute wound, in which neutrophils and macrophages have largely resolved from the wound site by 4dpw and 10dpw, respectively.²²

Figure 1. Suture implantation in adult zebrafish, collagen deposition, and number of innate immune cells surrounding implanted suture over time. (A) A polypropylene suture was implanted through the whole tail of the zebrafish (red X for entrance, black arrow, and green X for exit, dotted lines indicate where suture was embedded in the tissue, while solid lines indicate where the suture was outside of the tissue). (B) Representative gross view images, 7dpi. Scale bars are 1mm. (C-F) Multiphoton live microscopy z-stack projection of maximum intensity of individual transgenic adult (*Tg(mpx:GFP, mpeg1:mCherry)*) with neutrophils (green) and macrophages (red) separated from the second harmonics generation channel, which shows collagen in white, at 10dpi and 21dpi. Scale bars are 100μm and 'S' marks the site of the suture. (G-K) Collagen deposition via Masson's Trichrome stain, collagen in blue, nuclei in black, and muscle and cytoplasm in pink/purple. Scale bars are 30μm. (Q) Quantification

of collagen capsule thickness. n.s. is not significant, ++p<0.01, +++p<0.001 compared to the 1hpi time point and *p<0.05, **p<0.01, ***p<0.001, determined using a one-way ANOVA and Tukey's post-hoc multiple comparisons test. (L-P) Innate immune cells stained for L-plastin (green) and nuclei labeled with DAPI (blue). Scale bars are 30µm. (O) Quantification of number of L-plastin+ cells within the surrounding region 50µm from the outer edge of the suture, where *p<0.05, **p<0.01, ***p<0.001 compared to the 1hpi time point via a one-way ANOVA and Tukey's post-hoc multiple comparisons test. Dashed circles indicate location of suture, which was sometimes washed away during the sectioning/staining process, n≥3 zebrafish per time point.

The inflammatory response to polypropylene monofilament sutures or meshes, characterized by thin collagenous encapsulation,²⁷ increased macrophage infiltration,³³ macrophage fusion into foreign body giant cells,¹ and increased enzymatic activity surrounding the suture,³⁴ has been well-documented over the last 40 years in a variety of mammalian models including rat, porcine, rabbit, canine, and feline models.³⁵ The prolonged increase in collagen deposition and unchanging number of immune cells surrounding the suture observed in our study is quite distinct from the acute zebrafish wound healing response,²² and suggests that the host response to polypropylene sutures in adult zebrafish may resemble a traditional foreign body response. Interestingly, we did not observe the fusion of macrophages into multinucleated foreign body giant cells during histological or immunohistochemical analysis, but further research using high resolution microscopy will be required to confirm this finding.

To facilitate exploration of host-biomaterial interactions live and over time, a small pilot study was conducted to examine the response of immune cells to as a function of size of non-degradable polystyrene microparticles, another material known to elicit a foreign body response in mammals.¹² Microparticles (1µm, 10µm, and 25µm in diameter) were microinjected in larval zebrafish using a micropipette under a dissection microscope (**Figure 2A-B**). Qualitative analysis of videos showed that 1µm microparticles were phagocytosed by macrophages and were subsequently carried throughout the wound site by the macrophages (**Figure 2C-D; Movie S1**), while 10µm microparticles were surrounded by macrophages and appeared to be pushed around the wound site without being phagocytosed (**Figure 2E-F; Movies S2 & S3**). A single 25µm microparticle exhibited little, if any, movement within the wound site, but we observed neutrophil and macrophage contact with the particle (**Figure 2G-H; Movie S4**). These results indicate that immune cells displayed at least three unique responses in response to

microparticles of different sizes and illustrate the importance of macrophages and neutrophils in the body's response to a foreign material within larval zebrafish, as is observed in mammals.¹

Figure 2. Microparticle injection into transgenic larval zebrafish (A) Larval transgenic zebrafish (*Tg(mpx:GFP, mpeg1:mCherry)*, 4dpf) were microinjected with either 1µm (~20-30 microparticles), 10µm (~5-15 microparticles), or 25µm (one microparticle) polystyrene microparticles (yellow particles) ($n=1$ fish for 1µm and 10µm microparticles and $n=5$ fish for the 25µm microparticles). (B) Representative brightfield confocal microscopy image of a 25µm microparticle captured immediately following implantation. (C-D) Representative images from movies of a larval zebrafish implanted with 1µm polystyrene microparticles. Note that the representative image from the 1µm microparticle group was taken with a triple transgenic fish, *Tg(fli1:GFP, mpx:GFP, mpeg1:mCherry)*, in which macrophages are labeled red and both neutrophils and endothelial cells are labeled green. (E-F) A larval zebrafish implanted with 10µm polystyrene microparticles. (G-H) Larval zebrafish implanted with a single 25µm polystyrene microparticle. All images are from zebrafish 3dpi, represented as a z-stack projection of maximum intensity, and scale bars are 50µm. See also **Movies S1-S4**.

To observe temporal effects on immune cell behavior in real time, we created a simple model immunomodulatory biomaterial by adsorbing IL10 to 25µm polystyrene microparticles (**Figure 3A**). IL10 is an immunomodulatory cytokine involved in several important processes including the regulation of neutrophil recruitment to injury,³⁶ neutrophil phagocytic and bactericidal behavior,³⁷ and macrophage phenotype.³⁸ Therefore, we hypothesized that an IL10-releasing microparticle might influence neutrophil and macrophage behavior *in vivo*. IL10 release from microparticles exhibited classic desorption kinetics *in vitro* over 24 hours, characterized using enzyme-linked immunosorbent assay (ELISA).

Released protein was no longer detectable after the 8-hour time point (one-way ANOVA, with a post hoc Dunnett’s multiple comparison test against the zero control, $p>0.05$) and so the release study was terminated at that time (**Figure 3B**). A single IL10-releasing microparticle was then microinjected into larval zebrafish, and the behavior of fluorescent neutrophils and macrophages was monitored over time via live confocal imaging in comparison to an untreated microparticle control, a sham-wounded control, and an unwounded control. The *in vivo* study was conducted over 7 days to determine if any early effects from the IL10 release would have lasting effects on neutrophil or macrophage behavior. The wounded control and implantation of the untreated microparticle appeared to initially recruit similar numbers of macrophages and neutrophils, while the IL10 microparticle recruited noticeably fewer neutrophils at 1dpi (**Figure 3D, K, O; Movie S5**). Subsequently, there appeared to be no differences in macrophage or neutrophil numbers between the wounded control, untreated microparticle or IL10 microparticle in the 3, 5, or 7dpi time points (**Figure 3H-J, L-N, P-R**).

Figure 3. *IL10* Microparticle fabrication, characterization, and evaluation. (A) 25µm microparticles were physically adsorbed with recombinant zebrafish IL10 by overnight rocking incubation at 4°C. (B) Cumulative release of recombinant human IL10 via ELISA, per microparticle was analyzed over 24 hours *in vitro* (n=5). Transgenic larval zebrafish were microinjected with an IL10-adsorbed polystyrene microparticle (IL10 microparticle), non-treated polystyrene microparticle (untreated microparticle), or a sham injury into the dorsal somite directly opposite the cloaca and monitored over time (all groups and time points are n≥3, except for the untreated microparticle at 7dpi and IL10 microparticle at 5dpi, which are n=2). Raw data for each group and every time point are available in **Table S1**. Representative images of live confocal analysis are presented as z-stack projections of maximum intensity, (C-F) unwounded and (G-J) sham wounded controls, (K-N) untreated microparticles, and (O-R) IL10 microparticles. Scale bars are 50µm.

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Quantitative analysis of cell counts from images taken at each time point illustrated that the IL10 microparticle inhibited neutrophil recruitment surrounding the microparticle compared to the untreated microparticle after 1dpi (**Figure 4A**). These results are in keeping with previous reports in which the delivery of IL10 reduced the number of neutrophils intratracheally in rats within 6 hours³⁹ or intravenously in humans within 18 hours.⁴⁰ The release of a viral vector for IL10 from collagen disks implanted subcutaneously in rats after 21 days also did not affect macrophage recruitment.⁴¹ By 7dpi, all groups were not significantly different from the unwounded control. We saw no differences in the number of macrophages surrounding the microparticle with or without IL10 compared to the untreated microparticle or wound control at any time point (**Figure 4B**), which implies that IL10 has a role outside of recruitment for macrophages, in agreement with previous reports.^{42, 43} The transient effects of IL10 release on neutrophils and macrophages *in vivo* were not unexpected considering that the release of IL10 plateaued at some point between 8 and 24 hours *in vitro*, although release profiles between the *in vitro* and *in vivo* environments likely differ.^{44, 45} Collectively, the results of this study and previous work illustrate the importance of IL10 in the host-biomaterial response and how its presence surrounding implanted biomaterials has the potential to modulate the immune cell-biomaterial interactions.⁴¹

Figure 4. Quantification of neutrophils and macrophages surrounding microparticles with and without adsorbed IL10, as well as wounded and unwounded controls. (A) Neutrophils and (B) macrophages were manually counted within a concentric circle 50 μ m from the outside of the microparticle in Fiji. Samples size, n=2-7 per time point per group. Data were analyzed using two-way ANOVA and Tukey's post-hoc multiple comparisons test, **p<0.01 (untreated microparticle vs. IL10 microparticle), n.s. = not significant.

Surprisingly, the number of macrophages and neutrophils surrounding implanted microparticles in larval zebrafish decreased to baseline by 7dpi, whereas they remained elevated in response to polypropylene sutures in adult zebrafish. These divergent results may indicate a greater degree of foreign body response to polypropylene compared to polystyrene, or differences in immune cell behavior in larval and adult zebrafish. The latter is not entirely unexpected as it is known that the mammalian fetal response to injury is characterized by a more regenerative response than adults⁴⁶ and larvae lack an adaptive immune system.⁴⁷ Thus, larval zebrafish may represent a model for evaluating a regenerative response to biomaterials, but further analysis will be required to explore the potential for this application.

While zebrafish represent an attractive model for real time analysis of the foreign body response to biomaterials, they are not without limitations. First, the foreign body response is typically associated

with poor vascularization, but it is difficult to quantify blood vessel density in zebrafish. Quantifying the number of vascular structures using *in situ* hybridization and immunohistochemical techniques is possible⁴⁸ but not typically performed due to limitations in the specificity of vascular markers and transgenic models.⁴⁸⁻⁵¹ It is possible to inject resins into the bloodstream to visualize the vascular network via scanning electron microscopy, but resin fixation digests all local surrounding structure,⁵¹ destroying the fibrous capsule surrounding biomaterials and thus limiting spatial information. Moreover, this technique is technically challenging⁵¹ and increases the number of animals required. Consequently, a combination of complex techniques and crossed transgenic models will be required to characterize the number of vascular structures, and therefore was not explored in this study. Future work will explore the use of crossed pan-endothelial cell transgenic zebrafish lines, such as *Tg(fli1:eGFP)^{y152}* or *Tg(tie2:eGFP)*,⁵³ to enable dynamic live imaging of the 3D vascular and microvascular networks surrounding an implanted biomaterial. Secondly, markers of macrophage subtypes in zebrafish have not yet been established like they have been in other species,^{38, 54} although these transgenic fish are in development. For example, a tumor necrosis factor- α (TNF α) reporter line has been developed, (*Tg(tnfa:eGFP)*).⁵⁵ Crossing this line with a myeloid-specific line may enable visualization of pro-inflammatory “M1-like” macrophages, which express inflammatory cytokines like TNF α ,^{54, 56} but future work will be necessary to clearly identify and label macrophage phenotype-specific genes in zebrafish. Similarly, another zebrafish line that should enhance analysis of the foreign body response is the *Tg(mpeg1:mCherryCAAX)sh378*, in which macrophages and their membranes are labeled;⁵⁷ the use of this line may enable high resolution structural analysis of foreign body giant cells, if they do exist in zebrafish. Finally, potential methods of implanting more complex biomaterials may include intraperitoneal injection or development of a flap model in which scales and skin on the flank of the fish are dissected to create a pocket.

In summary, this preliminary study illustrates that adult zebrafish exhibit evidence of host-biomaterial interactions that resemble a foreign body response, and larval zebrafish provide a facile

window to explore the dynamics of immune cell behavior in response to immunomodulatory biomaterials in real-time. Future work will be essential for advancing this novel model includes analysis of other diverse biomaterials to ensure that material-specific differences in immune cell behavior can be discerned. With further characterization, this model has potential to allow higher throughput investigation of immune cell-biomaterial interactions *in vivo* in clinically relevant models.

SUPPORTING INFORMATION

The Supporting Information is available free of charge on the ACS Publications website.

Detailed experimental methods and supplementary movie captions (PDF)

[Figure S1: Gross anatomy of adult zebrafish controls or implanted with suture over time](#)

Movie S1 showing 1 μ m microparticles, 3dpi (AVI)

Movie S2 showing 10 μ m microparticles, 3dpi (AVI)

Movie S3 showing 10 μ m microparticles, 3dpi, zoomed (AVI)

Movie S4 showing a 25 μ m untreated microparticle, 3dpi (AVI)

Movie S5 showing a 25 μ m IL10 microparticle, 3dpi (AVI)

[Table S1: Quantification of neutrophils and macrophages around 25 \$\mu\$ m microparticle over time](#)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no conflicts of interest.

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Figure 1. Suture implantation in adult zebrafish, collagen deposition, and number of innate immune cells surrounding implanted suture over time. (A) A polypropylene suture was implanted through the whole tail of the zebrafish (red X for entrance, black arrow, and green X for exit, dotted lines indicate where suture was embedded in the tissue, while solid lines indicate where the suture was outside of the tissue). (B) Representative gross view images, 7dpi. Scale bars are 1mm. (C-F) Multiphoton live microscopy z-stack projection of maximum intensity of individual transgenic adult (Tg(mpx:GFP, mpeg1:mCherry)) with neutrophils (green) and macrophages (red) separated from the second harmonics generation channel, which shows collagen in white, at 10dpi and 21dpi. Scale bars are 100μm and 'S' marks the site of the suture. (G-K) Collagen deposition via Masson's Trichrome stain, collagen in blue, nuclei in black, and muscle and cytoplasm in pink/purple. Scale bars are 30μm. (Q) Quantification of collagen capsule thickness. n.s. is not significant, ++p<0.01, +++p<0.001 compared to the 1hpi time point and *p<0.05, **p<0.01, ***p<0.001, determined using a one-way ANOVA and Tukey's post-hoc multiple comparisons test. (L-P) Innate immune cells stained for L-plastin (green) and nuclei labeled with DAPI (blue). Scale bars are 30μm. (O) Quantification of number of L-plastin+ cells within the surrounding region 50μm from the outer edge of

the suture, where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the 1hpi time point via a one-way ANOVA and Tukey's post-hoc multiple comparisons test. Dashed circles indicate location of suture, which was sometimes washed away during the sectioning/staining process, $n \geq 3$ zebrafish per time point.

Figure 1
241x285mm (300 x 300 DPI)

Figure 2. Microparticle injection into transgenic larval zebrafish (A) Larval transgenic zebrafish (*Tg(mpx:GFP, mpeg1:mCherry)*, 4dpf) were microinjected with either 1µm (~20-30 microparticles), 10µm (~5-15 microparticles), or 25µm (one microparticle) polystyrene microparticles (yellow particles) ($n=1$ fish for 1µm and 10µm microparticles and $n=5$ fish for the 25µm microparticles). (B) Representative brightfield confocal microscopy image of a 25µm microparticle captured immediately following implantation. (C-D) Representative images from movies of a larval zebrafish implanted with 1µm polystyrene microparticles. Note that the representative image from the 1µm microparticle group was taken with a triple transgenic fish, *Tg(fli1:GFP, mpx:GFP, mpeg1:mCherry)*, in which macrophages are labeled red and both neutrophils and endothelial cells are labeled green. (E-F) A larval zebrafish implanted with 10µm polystyrene microparticles. (G-H) Larval zebrafish implanted with a single 25µm polystyrene microparticle. All images are from zebrafish 3dpi, represented as a z-stack projection of maximum intensity, and scale bars are 50µm. See also Movies S1-S4.

Figure 2

171x165mm (300 x 300 DPI)

Figure 3. IL10 Microparticle fabrication, characterization, and evaluation. (A) 25µm microparticles were physically adsorbed with recombinant zebrafish IL10 by overnight rocking incubation at 4°C. (B) Cumulative release of recombinant human IL10 via ELISA, per microparticle was analyzed over 24 hours in vitro (n=5). Transgenic larval zebrafish were microinjected with an IL10-adsorbed polystyrene microparticle (IL10 microparticle), non-treated polystyrene microparticle (untreated microparticle), or a sham injury into the dorsal somite directly opposite the cloaca and monitored over time (all groups and time points are n≥3, except for the untreated microparticle at 7dpi and IL10 microparticle at 5dpi, which are n=2). Raw data for each group and every time point are available in Table S1. Representative images of live confocal analysis are presented as z-stack projections of maximum intensity, (C-F) unwounded and (G-J) sham wounded controls, (K-N) untreated microparticles, and (O-R) IL10 microparticles. Scale bars are 50µm.

Figure 3
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Figure 4. Quantification of neutrophils and macrophages surrounding microparticles with and without adsorbed IL10, as well as wounded and unwounded controls. (A) Neutrophils and (B) macrophages were manually counted within a concentric circle 50 μ m from the outside of the microparticle in Fiji. Samples size, n=2-7 per time point per group. Data were analyzed using two-way ANOVA and Tukey's post-hoc multiple comparisons test, **p<0.01 (untreated microparticle vs. IL10 microparticle), n.s. = not significant.

Figure 4
105x134mm (300 x 300 DPI)