Purinergic Signaling in Early Inflammatory Events of the Foreign Body Response: Modulating Extracellular ATP as an Enabling Technology for Engineered Implants and Tissues

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Purinergic signaling is a ubiquitous and vital aspect of mammalian biology in which purines—mainly adenosine triphosphate (ATP)—are released from cells through loss of membrane integrity (cell death), exocytosis, or transport/diffusion across membrane channels, and exert paracrine or autocrine signaling effects through three subclasses of well-characterized receptors: the P1 adenosine receptors, the P2X ionotropic nucleotide receptors, and the P2Y metabotropic receptors. ATP and its metabolites are released by damaged and stressed cells in injured tissues. The early events of wound healing, hemostasis, and inflammation are highly regulated by these signals through activation of purinergic receptors on platelets and neutrophils. Recent data have demonstrated that ATP signaling is of particular importance to targeting leukocytes to sites of injury. This is particularly relevant to the subject of implanted medical devices, engineered tissues, and grafts as all these technologies elicit a wound healing response with varying degrees of encapsulation, rejection, extrusion, or destruction of the tissue or device. Here, we review the biology of purinergic signaling and focus on ATP release and response mechanisms that pertain to the early inflammatory phase of wound healing. Finally, therapeutic options are explored, including a new class of peptidomimetic drugs based on the ATP-conductive channel connexin43.

Introduction

THE INJURY RESPONSE, including sterile surgical wounds, is an integral consideration for any tissue-engineered repair. It has been suggested that in tissue engineering, many problems can be traced back to scarring at the culmination of the healing process.¹ Injury produces a complex and highly orchestrated sequence of events as the body attempts to restore tissue to its preinjury state.² Wound healing is divided into three overlapping, well-choreographed, event-driven phases: hemostasis-inflammation, proliferation, and scarring-remodeling.^{3,4} Normal resolution of inflammation in small defects results in low levels of scarring and essentially restored tissue function and histology. Larger wounds can transition to chronic inflammatory states, which in turn tends to produce more scarring and a concomitant loss of normal tissue histoarchitecture and function.^{5,6}

Surgical engraftment of implanted devices, tissues, and engineered constructs into the body will elicit an injury response, including acute inflammation, tissue remodeling, and scarring at the site of implantation. This multifaceted response involves the participation of a number of inflammatory and fibrotic cell types including mast cells, neutro-

phils, monocytes/macrophages, lymphocytes, and fibroblasts.⁷ In particular, long-term, chronic inflammation mediated by macrophages has been an active area of recent research (see McNally and Anderson for an excellent review⁸). Activated macrophages adhere to implanted biomaterials and fuse to form multinucleated foreign body giant cells that persist at the implant site and promote granulation tissue formation, eventually leading to fibrous encapsulation.^{7,8}

Complicating this is the issue of macrophage “polarization.” Macrophages can assume multiple phenotypes that can be broadly characterized as M1 “classically activated” or M2 “alternatively activated” phenotypes. In general, M1 macrophages are proinflammatory, while M2 macrophages are anti-inflammatory and promote regeneration (this topic is extensively reviewed by Brown *et al.*⁹). Uniquely, this review will focus on the equally important initial events that lead to chronic inflammation and the formation of a fibrotic capsule—namely, hemostasis and the neutrophil response.¹⁰

A number of paracrine/autocrine signaling pathways are known to initiate, regulate, and dampen the inflammatory response—primarily (anti-)inflammatory cytokines such as interleukin-6 (IL-6), IL-8, macrophage inflammatory protein-2 (MIP-2), and IL-10. However, recent research has

brought to light the key role that an alternative signaling pathway plays in the inflammatory response. Namely, purinergic signaling, or signaling through extracellular nucleotides/sides, is involved in hemostasis and recruitment of inflammatory cell types.

The primary mediator in purinergic signaling is adenosine triphosphate (ATP), which is a biological molecule most commonly thought of for its roles in intracellular energy transfer and as a precursor for deoxyribonucleic acid (DNA). Further, ATP is at the forefront of biology as a substrate for kinase reactions in signal transduction pathways, and adenylate cyclase, which converts ATP to the second messenger cyclic adenosine monophosphate (AMP). However, evidence accumulated over the past 40 years has established that ATP can also act as a neurotransmitter and paracrine/autocrine signaling molecule.¹¹

In mammalian systems purinergic signaling is a crucial component of numerous (patho)physiological phenomena, including synaptic transmission,¹² regulation of vascular tone,¹³ inner ear function and hearing,¹⁴ cell volume regulation,¹⁵ ischemic responses in the heart¹⁶ and brain,¹⁷ propagation of intercellular calcium waves,^{18,19} the spread of apoptotic cell death,²⁰ kidney function,²¹ hypoxic preconditioning,²² Alzheimer's Disease,²³ cell proliferation,²⁴ memory,²⁵ cancer,²⁶ embryonic development,²⁷ nociception,²⁸ and inflammatory diseases.²⁹ In addition to these many roles for ATP, recent data indicate a crucial role for extracellular ATP in inflammatory cell targeting to sites of injury.^{30–33} The focus of this review will be purinergic signaling in the context of injury and the resulting acute inflammation, and how this affects the host response to implanted devices and engineered tissues. Although little data exist on the role of extracellular ATP in macrophage polarization, for readers interested in ATP signaling in chronic inflammation Lemaire *et al.* provide a first-rate review of purinergic signaling in the process of giant cell formation.³⁴ The purpose of this review is to communicate the importance of this largely ignored signaling mechanism to the tissue engineering and surgical implant community.

Basic Biology of Purinergic Signaling

ATP release mechanisms

Cell death. ATP exists in the cytoplasm of cells at high concentrations, ranging from 1–10 mM.³⁵ Therefore, one of the most common and important mechanisms of ATP release is simply cell rupture. Given that some purinergic receptors have an EC₅₀ for ATP as low as 1 μ M it is clear that rupture of even a small number of cells can elicit a response in nearby viable cells.^{13,36}

Exocytosis. ATP has also been shown to be present in synaptic vesicles.^{37,38} Evidence that ATP was released by exocytosis of synaptic vesicles first came from experiments in which exogenously applied quinacrine, a fluorescent antimalarial known to bind ATP,³⁹ was observed to decrease in the cytoplasm of nerves undergoing depolarization.⁴⁰ Recent work by Sawada *et al.* has identified a protein termed the vesicular nucleotide transporter (VNUT) that they demonstrated to mediate vesicular ATP uptake.⁴¹ Importantly, they showed that PC12 cells, reported to secrete ATP through exocytosis,⁴² displayed reduced ATP release

when treated with VNUT siRNA,⁴¹ strongly indicating that exocytosis is a viable mechanism of ATP release. Importantly, a number of other cell types, including endothelial cells, display exocytosis of ATP.^{43,44} Endothelial cells are of particular interest for this review because of their access to the blood vessel lumen from which inflammatory cells can be recruited to sites of injury, implanted devices, or tissues.

ABC transporters. ATP-binding cassette (ABC) transporters are a superfamily of membrane proteins that hydrolyze ATP to power the unidirectional efflux of a large variety of molecules.⁴⁵ Perhaps the most well-known of the ABC transporters is the chloride ion transporter, cystic fibrosis transmembrane conductance regulator (CFTR), mutations of which are responsible for the cystic fibrosis genetic disorder.⁴⁶ In addition to other ABC transporters, the CFTR has also been shown to mediate outward ATP currents.⁴⁷ This mechanism of ATP release potentially plays an important role in injured tissues as the CFTR is expressed in a number of relevant cell types including vascular endothelial cells,⁴⁸ red blood cells,⁴⁹ platelets,⁵⁰ and skeletal muscle.⁵¹

Connexin hemichannels. Connexins are a family of integral membrane proteins that form intercellular channels located in plaque-like structures called gap junctions (GJs) that form at points of cell–cell contact.⁵² These channels facilitate the diffusion of small molecules (< ~1000 Da) and ions between cells. The intercellular channels are formed by the interaction of two half-channels (one from each cell), called hemichannels.

Hemichannels newly delivered to the membrane are not immediately incorporated into the GJs of contacting cells, but reside in an adjacent region of the membrane called the perinexus where they await incorporation into the periphery of the GJ plaque.^{53–55} While at the perinexal plasma membrane, hemichannels create a conduit for passive diffusion between the cytoplasm and extracellular space.⁵³ ATP release through connexin hemichannels has been demonstrated in a number of cell types, and under a number of conditions, displaying both physiological and pathological properties,⁵⁶ and in particular, this review will focus on Cx43-mediated ATP release from hemichannels in neutrophils and endothelial cells, with relevance to inflammation.

Pannexin channels. Pannexin channels are vertebrate homologues of the invertebrate GJ proteins, innexins, but are phylogenetically unrelated to connexins.⁵⁷ Unlike connexins, pannexins do not form intercellular channels or GJs; rather, they exist in the plasma membrane as channels that can connect the cytoplasm to the extracellular space.⁵⁸ Early patch clamp studies demonstrated the permeability of pannexin channels to ATP, and indicated the potential for mechanosensitive ATP release.⁵⁹

Breakdown of extracellular ATP

It may seem counterintuitive to discuss the removal of extracellular ATP prior to reviewing the receptors for ATP, but the breakdown products of ATP (adenosine diphosphate [ADP], AMP, and adenosine) are themselves agonists of purinergic receptors. ATP released into the extracellular space by any of the above mechanisms is rapidly degraded

by ecto- and exo-nucleotidases, which are cell-surface attached and soluble in the extracellular space, respectively. These enzymes catalyze the breakdown of ATP to ADP and AMP, and further catalyze the breakdown of AMP to adenosine and inorganic phosphate.

Of the ectonucleotidases, CD39 and CD73 are most salient to this discussion. CD39 (also known as ectonucleotide triphosphate diphosphohydrolase 1) is expressed by a number of cell types including vascular endothelial cells,⁶⁰ leukocytes,⁶¹ and lymphocytes.⁶² It catalyzes the breakdown of ATP to ADP, and further catalyzes the hydrolysis of ADP to AMP.⁶⁰ AMP is further hydrolyzed to adenosine by ecto-5'-nucleotidase, or CD73.⁶³ Like CD39, CD73 is expressed on a number of cell types including endothelial cells⁶¹ and neutrophils.⁶⁴ Its expression appears to modulate endothelial cell barrier function⁶³ and leukocyte adhesion,³⁰ mediated through the generation of adenosine. Finally, adenosine is deaminated to inosine by adenosine deaminase, effectively removing the molecule from purinergic signaling pathways.⁶⁵

Purinergic receptors

Once ATP is released from cells, it can have paracrine/autocrine signaling effects through purinergic (P) receptors. These receptors can be broadly grouped into P1 and P2 families, which are activated by adenosine and primarily ATP/ADP, respectively.¹¹ The P2 receptors can be broken down again into 2 main subtypes, P2X and P2Y, which are ligand-gated ion channels and G-protein coupled receptors (GPCRs), respectively.¹¹

P1. P1 receptors are GPCRs that are sensitive to the nucleoside adenosine. There are currently four known P1 receptors: A₁, A_{2A}, A_{2B}, and A₃.⁶⁶

P2X. Unlike the P1 and P2Y GPCRs, P2X receptors are ligand-gated trimeric ion channels.²⁸ Once activated by ATP, the channel opens within milliseconds and forms a selective cation pore with a strong preference for Ca²⁺ followed by Na⁺ and K⁺.²⁸ Pore opening generally results in membrane depolarization, but also an increase in intracellular Ca²⁺ levels, activating differing second messenger systems depending on cell type.⁶⁷ For example, in macrophages, P2X₇ expression is important for release of the proinflammatory cytokine IL-1 β .⁶⁸ It has been demonstrated that this effect is mediated by ATP activation of P2X₇ receptors, and subsequent activation of the Nlrp3 inflammasome, a multiprotein complex that activates caspase-1 resulting in the processing and secretion of the neutrophil chemoattractant IL-1 β .⁶⁹

P2Y. Like P1 receptors, P2Y receptors are structurally similar GPCRs. There are currently 8 known subtypes: P2Y₁, P2Y₂, P2Y₄, P2Y₆, and P2Y₁₁₋₁₄.⁶⁶ Interestingly, P2Y receptors not only respond to ATP, but also display mixed sensitivities to ADP, UTP, and UDP depending on cellular and tissue context.⁷⁰

Purinergic Signaling and Early Inflammatory Events in Injured Tissues

The sequence of events following injury to any tissue—whether the cause is burns, lacerations, infarct, or surgical

placement of an implant—is basically the same.⁷¹⁻⁷³ The initial response includes hemostasis (if there is injury to blood vessels) and infiltration of the wounded tissue by neutrophils.⁷¹ The neutrophil response in particular has been associated with poor outcomes, and several lines of evidence, including studies in neutropenic mice,⁷⁴ embryonic healing,⁷⁵ and with modulation of Cx43,^{4,76,77} indicate that reducing neutrophil invasion leads to improved restoration of original tissue architecture (or reduction of fibrous capsule formation, in the case of implants⁷⁸) as opposed to replacement by scar. Therefore, we review these events, and the role purinergic signaling plays in them, in the following sections (also see Fig. 1).

Early inflammatory events

Hemostasis. Following an injury that damages a blood vessel, platelets adhere and aggregate forming a plug enmeshed in a fibrin clot formed from fibrinogen cleavage by thrombin.⁷⁹ The clot serves several purposes. First, the clot restores hemostasis, preventing further blood loss.⁸⁰ Second, the platelets secrete cytokines with chemotactic and mitogenic properties—platelet-derived growth factor, epidermal growth factor, and transforming growth factors β 1 and β 2.⁸⁰ The neutrophils, macrophages, and fibroblasts that respond to these signals are able to migrate into the wound because of the third function of the thrombus, which is to provide a temporary extracellular matrix for cell migration.⁸⁰

Purinergic signaling is important for the process of platelet aggregation. ADP generated from damaged tissue has long been recognized to induce platelet aggregation.^{81,82} ADP is also secreted by platelets themselves by degranulation,^{83,84} potentially forming a positive feedback loop.⁸⁵

Purinergic signaling also functions to contain coagulation to the site of injury. ATP acts as an antagonist of platelet P2Y receptors and functions to limit thrombus formation.⁸⁶ Recent work has demonstrated ATP release from vascular endothelial cells in response to thrombin.⁸⁷ Using pharmacological inhibitors of connexins and pannexins and shRNA technology, the authors provide compelling evidence that pannexins are the channel responsible for ATP release in response to thrombin. This response to thrombin in vascular endothelial cells suggests that ATP may be released in an injury-localized manner that serves to locally limit platelet aggregation to areas of vessel damage and simultaneously attract inflammatory cells such as neutrophils. Taken together, these data indicate that purinergic signaling plays an important role in both activating and regulating hemostasis.

The neutrophil response. Neutrophils are the next cellular component to enter the wound area, and arrive within minutes of injury.⁸⁸ Their main role is to cleanse the area of debris and bacteria.⁸⁰ They also serve as a source of proinflammatory cytokines activating local fibroblasts and keratinocytes.⁸⁹ Ironically, neutrophils can also cause extensive damage to the tissue through the release of reactive oxygen species (ROS).⁹⁰ Neutrophils are eventually removed from the wound with the eschar and phagocytosed by macrophages.⁸⁴

Recent data have implicated a role for injury-generated ATP in the production of chemoattractants, and subsequent targeting of neutrophils to sites of sterile inflammation.

FIG. 1. Purinergic signaling in inflammation. This illustration depicts some of the key pathways in which purinergic signals mediate the initial inflammatory response. Adenosine triphosphate (ATP) can be released in a number of ways: damaged cells in injured tissue spill ATP and adenosine diphosphate (ADP) into the extracellular space through loss of membrane integrity; tissue compression can stimulate ATP release from endothelial cells through connexin hemichannels by direct mechanical stimulation and changes in shear stress; mechanically stimulated myocytes release ATP through the cystic fibrosis transmembrane conductance regulator and pannexin channels; endothelial cells release ATP through pannexin channels in response to thrombin. Once released, ATP acts through P2Y₂ and P2X₁ receptors on neutrophils to simulate migration. ATP also acts at P2X₇ receptors on resident macrophages, which activates the Nlrp3 inflammasome resulting in macrophage inflammatory protein-2 (MIP-2) and interleukin (IL)-8 release, which stimulate neutrophil migration and promote neutrophil–endothelial cell interactions respectively. Platelet aggregation is limited by ATP, while ADP from dead cells and activated platelets stimulates aggregation through P2Y₁ and P2Y₁₂. Finally, ATP is converted to adenosine monophosphate (AMP) by CD39, which is in turn converted to adenosine by CD73. Adenosine at low concentrations promotes neutrophil migration through A₃, but at higher concentrations acts through A_{2A} and A_{2B} to inhibit migration. This may be a mechanism to prevent extravasated neutrophils from returning to the vasculature.

McDonald *et al.* generated a thermal injury on the liver, and found that neutrophils migrated directionally to the site of injury.³¹ Their data suggested that ATP in the injury area activated P2X₇ receptors on resident macrophages, which in turn activated the Nlrp3 inflammasome. This led to the

production of MIP-2 and IL-8, targeting neutrophils to the wound site and promoting endothelial cell/neutrophil interaction through integrin $\alpha_M\beta_2$ (MAC1) on neutrophils and intercellular adhesion molecule-1 on endothelial cells. Once neutrophils were near the wound locus, migration into the

injury appeared to be mediated by formylated peptides released from dead cells.

Interestingly, McDonald *et al.* also found that ATP did not directly mediate neutrophil migration, in contrast with the published literature.^{31,33} Specifically, Chen *et al.* have shown that neutrophils directionally migrate toward fMLP in an ATP-dependent manner.³³ They found that fMLP elicited polarized ATP release from neutrophils, generating an ATP concentration gradient that the neutrophils could follow. More recent work from this group has demonstrated that the route of ATP release was through pannexin channels.⁹¹ However, others have demonstrated that Cx43 hemichannels are a conduit for ATP in activated neutrophils.⁶¹

In addition to ATP, adenosine also modulates a number of neutrophil responses. A₁ and A₃ receptors generally act through G_{i/o} signaling while the A_{2A} and A_{2B} receptor subtypes signal through the counteracting G_s system. Importantly, A₁ and A₃ receptors have a high affinity for adenosine (EC₅₀ < 500 nM), while A_{2A} receptors have lower affinities, and A_{2B} receptors have EC₅₀'s > 10 μ M.⁹² These opposing pathways play an important role in early inflammatory events. Specifically, A₁ and A₃ receptor activation in neutrophils largely lead to proinflammatory actions: enhanced chemotaxis, adhesion, phagocytosis, and superoxide production. Conversely, pathways activated by A_{2A} and A_{2B} inhibit adhesion, migration, degranulation, inflammatory cytokine release, and ROS production.⁹²

Stimulus and sources of ATP and adenosine in the wound healing response

In the setting of an injury, cell necrosis and apoptosis likely represent an acute infusion of ATP into the system.³¹ This source of ATP appears to be important for recruiting neutrophils, but this alone may be insufficient to stimulate a full response. Extracellular ATP is quickly degraded by ectonucleases,⁹³ and therefore secondary sources of ATP are necessary for continued signaling. These secondary sources appear to be leakage from connexin hemichannels^{19,94} and pannexin channels⁵⁹ in response to mechanical¹¹ and flow induced shear stress.^{95,96} For example, in a brain injury model, work by Davalos and colleagues demonstrated that insertion of an electrode containing ATP into the cerebral cortex was enough to elicit a microglial response, but the response abruptly ended upon removal of the pipette.³² In an elegant experiment, they further showed that ATP-induced ATP release was necessary for the microglial response. This was accomplished by applying the P2Y agonizing nonhydrolyzable ATP analogue AMPPNP in the presence of apyrase, an exonucleotidase. In this condition, or with connexin inhibitors, no microglial response was observed, indicating that continuing microglial activation was due to P2Y-stimulated ATP release through connexin channels.

Implanted devices and ATP

The initial hemostatic and inflammatory response to the surgical placement of an implant, biomaterial, or transplanted tissue are key factors mediating the foreign body response,^{72,97} or rejection of transplants and grafts.^{98,99} In the case of implants, those early inflammatory events recruit macrophages and fibroblasts that encapsulate the implant

and create contractile tissue, potentially leading to physical disfigurement, discomfort, device malfunction, and/or extrusion of the implant; for allo- or xenogeneic transplanted tissues, changes in the microvasculature and/or vascular thrombosis can cause ischemia of the introduced tissue leading to cell death and rejection.

Little data currently exist directly linking purinergic signaling to negative outcomes in the foreign body response to implants. However, we have observed that application of apyrase to the implant pocket of silicone discs placed sub-muscularly markedly reduces infiltration of inflammatory cells into the surrounding musculature (Fig. 2). Further, the initial inflammatory response to an implant is very similar to that of normal tissue injury, with the exception that encapsulation and chronic inflammation can occur with an implant.⁷² Therefore, much can be extrapolated from the studies discussed above relating purinergic signaling to the hemostatic and neutrophil responses in wounded tissue. We propose that neutrophils, blood clots, and associated platelets are activated by and targeted to implant sites by purinergic signals (Fig. 1). Further, these cells and the thrombus itself are known to release factors that attract macrophages and fibroblasts, which contribute to the formation of a contraction capsule around the implant and induce further inflammation in a chronic cycle.⁷²

Evidence supporting this hypothesis comes from work by Luttikhuisen *et al.*¹⁰⁰ The authors studied purinergic receptor expression following subcutaneous implantation of a collagen disk in rats. They found that P2X₇, P2Y₁, and P2Y₂ receptors displayed increased expression in the vasculature around the implant until day 2. Infiltrating macrophages and giant cells reacted differently, with expression of P2X₇ and P2Y₁ receptors increasing over the full period studied of 21 days. Importantly, these data suggest that in the setting of an implanted material the ability of cells to respond to ATP may be increased, potentially exacerbating negative outcomes.

Scaffold-based materials, cellularized constructs, and ATP

As is the case with synthetic implants, there is a dearth of data concerning the role of extracellular ATP in the response to scaffold-based materials. However, like other implantable materials, hydrogels based on collagen,^{101,102} hyaluronic acid,¹⁰¹ agarose,¹⁰¹ polyethylene glycol,¹⁰³ and alginates¹⁰⁴ generally elicit an innate immune response in the form of robust infiltration of neutrophils into the surrounding tissue, with subsequent encapsulation. Exceptions to this phenomenon are rare in the literature, but some cross-linked polyethers have been shown to have mild innate immune responses.¹⁰⁵ Further, we have found that aligned collagen constructs become populated by host cells and vascularized, as opposed to inducing the formation of a fibrotic capsule.¹⁰⁶

As with synthetic implants, the similarity of the acute inflammatory response to scaffold materials compared to normal tissue injury strongly suggests that purinergic signaling is involved. In support of this notion, recent work from David Becker's lab has shown that placement of a collagen scaffold in full thickness mouse wounds impeded reepithelialization and increased the number of infiltrating

FIG. 2. Apyrase reduces inflammation in an implant model. Silicone implants were submuscularly placed in the backs of Sprague-Dawley rats, and tissue was harvested from the implant site 24 h postsurgery. The tissue was sectioned and stained by hematoxylin and eosin. In (A) and (C) 200 μ L of vehicle (phosphate-buffered saline) was pipetted into the implant pocket along with the silicone disc—"Control"; in (B) and (D) 200 μ L of apyrase (25 U/mL) was pipetted into the implant pocket with the disc—"Apyrase." Asterisks indicate areas with necrosed bundles of muscle tissue. Note the preservation of muscle tissue in apyrase treated (B) animals compared to control (A) animals. Arrowheads point to areas where inflammatory cells have invaded the musculature and have begun to cause coagulative necrosis. White arrows show the direction of the implant. Scale bars = 500 μ m in (A) and (B), and 100 μ m in (C) and (D). Color images available online at www.liebertpub.com/teb

neutrophils.¹⁰² Importantly, they found that Cx43 was up-regulated at the leading edge of keratinocytes, suggesting the potential for increased release of proinflammatory signals such as ATP.

A major goal for implanting scaffold-based materials has been the delivery of cells—often pluripotent or stem cells—for the regeneration of injured tissue. It is a well-known phenomenon that delivery of "naked" cells into an animal is generally associated with poor survival of the implanted cells.^{107–109} Indeed, a recent study in which cardiac derived stem cells were intramyocardially injected into rats shows that a commonly used magnetic resonance imaging (MRI)-based method for detecting injected cells grossly overestimates cell survival, with few stem cells actually surviving at 3 weeks.¹¹⁰ Surprisingly, few studies have characterized cell survival in protocols using scaffold-seeded or -encapsulated cell delivery systems. Mayfield *et al.* delivered cardiac stem cells encapsulated in agarose to help repair an induced myocardial infarction in mice.¹¹¹ While delivery of encapsulated stem cells did improve cardiac function and reduce infarct size, and survival of encapsulated cells was significantly greater than nonencapsulated cells, the number of transplanted encapsulated cells remaining was only 20% at 1 h and 10% at 3 weeks postdelivery. In combination with the data on the innate immune response to scaffold-based materials, it can be inferred that just as the neutrophil response generates collateral damage to host tissues, neutrophils may also destroy a large number of implanted cells. Therefore, it is strongly suggested that loss of transplanted cells to the initial inflammatory response is a key issue for all researchers developing tissue-engineering methodologies that has been

largely overlooked, and the signaling mechanisms involved (e.g., extracellular ATP) should be investigated.

Therapeutic Options

A standard response to nondegradable synthetics is capsule formation and resultant capsular contracture.¹¹² This process can result in implant extrusion, device failure, disfigurement, and chronic pain.^{112,113} The interface of these materials and the host tissue never completes the wound healing cycle forming in essence a chronic granuloma.^{78,113} Further, prosthetic materials remain vulnerable to infection from bacteremia or direct seeding by infectious organisms. Therapeutic interventions that could temporize, lessen, or allow better engraftment of synthetic materials would be of tremendous clinical benefit saving millions in health care costs and more importantly, patient well being.

Apyrase as an ATP scavenger

Soluble apyrase converts ATP to AMP and is found throughout nature. A number of plant species express soluble apyrases,¹¹⁴ and apyrase isolated from potatoes is commercially available as a relatively inexpensive lyophilized powder. Animal studies show that application of apyrase improves outcomes in situations where the initial inflammatory response is undesirable. For example, infarct volumes, inflammatory cytokines, and numbers of infiltrating neutrophils were significantly greater in CD39-deficient mice compared with wild-type mice in a model of cerebral ischemia.⁶⁴ Application of soluble potato apyrase to CD39-deficient mice prior to ischemia significantly reduced infarct

volumes and neutrophil counts compared with saline-injected controls. Although apyrase administration to wild-type mice was not performed, these data indicate that apyrase is a potential therapeutic in cases of stroke, especially given its platelet disaggregating effects.

The utility of apyrase treatment is also supported in skin and liver injury. In a model of epidermal injury, Takahashi *et al.* used an innovative agarose bead-attached green-emitting luciferase to image subcutaneous ATP release.¹¹⁵ They found that following tape-stripping of mouse skin, ATP release was increased and its bioluminescent detection could be quenched by application of apyrase. Importantly, subepidermal neutrophil infiltrate was observed after injury, and could be significantly reduced by apyrase application. Similarly, in a mouse model of acetaminophen-induced hepatotoxicity, overdose resulted in liver necrosis and massive neutrophil infiltration; again, apyrase administration significantly attenuated this response.¹¹⁶ Apyrase treatment has also shown improvement of outcome and reduction of inflammation in a number of other models including lipopolysaccharide-induced lung inflammation,¹¹⁷ laryngeal airway hyperreactivity,¹¹⁸ asthma,¹¹⁹ mechanical ventilation-induced acute lung injury,¹²⁰ and cardiac xenograft.¹²¹

In the case of implants, we have observed reductions in inflammatory cell infiltrate and coagulative necrosis of muscle tissue when apyrase was applied in a rat submuscular implant model (Fig. 2). Taken together with the above discussed studies, it is suggested that inclusion of apyrase with implanted materials and devices could reduce the incidence of capsular contracture and extrusion.

Blocking or manipulating Cx43 hemichannels

As discussed above, connexin hemichannels and pannexin channels open in response to a number of pathological stimuli and are a conduit for ATP release in diverse scenarios. Manipulation of connexins has proved beneficial in wound healing and in the response to implants. Pioneering work by Qiu *et al.* demonstrated that application of Cx43 antisense to incisional and excisional skin wounds in mice accelerated wound closure, reduced scarring, and limited neutrophil infiltration of the wound area.⁴ Similar results have been produced using the Cx43-mimetic peptide α CT-1.⁷⁶ Notably, work from the Yost lab has shown that application of α CT-1 to submuscularly placed silicone implants in rats significantly reduced neutrophil infiltration in muscle tissue adjacent to the implant.⁷⁸ Additionally, there was reduced collagen content, increased vascularity, and a reduction of contractile myofibroblasts around the implant in α CT-1 treated rats—suggesting a significantly attenuated foreign body response. Importantly, α CT-1 has been shown to reduce hemichannel activity,⁵³ indicating that these effects could be partially mediated by a reduction in ATP release through Cx43 hemichannels.

Concluding Remarks

The field of purinergic signaling encompasses a wide range of phenomenon—from synaptic transmission to renal function to inflammation. This diversity of function is nothing short of amazing considering the completely separate and central functions of intracellular ATP in energy

metabolism and DNA structure. Yet even within the narrow scope of early inflammatory events the role that purinergic signaling plays is not easily defined, having multiple mechanisms of release and acting through different receptors depending on context and cell type, and at times acting through synergistic pathways or antagonistic pathways. We are only beginning to unravel the complex web, which is the cellular and molecular biology of purinergic signaling, but already we are beginning to see the development of therapeutics out of basic science research on extracellular ATP signaling. With this in mind, the prospects for enhancing healing and integration of foreign organs, synthetic tissues, and implanted devices are improving daily.

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References

- Atala, A., Bauer, S.B., Soker, S., Yoo, J.J., and Retik, A.B. Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet* **367**, 1241, 2006.
- Ashcroft, G.S., Yang, X., Glick, A.B., Weinstein, M., Letterio, J.L., Mizel, D.E., *et al.* Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nat Cell Biol* **1**, 260, 1999.
- Martin, P., D'Souza, D., Martin, J., Grose, R., Cooper, L., Maki, R., *et al.* Wound healing in the PU.1 null mouse—tissue repair is not dependent on inflammatory cells. *Curr Biol* **13**, 1122, 2003.
- Qiu, C., Coutinho, P., Frank, S., Franke, S., Law, L.Y., Martin, P., *et al.* Targeting connexin43 expression accelerates the rate of wound repair. *Curr Biol* **13**, 1697, 2003.
- Lumelsky, N.L. Commentary: engineering of tissue healing and regeneration. *Tissue Eng* **13**, 1393, 2007.
- Mountziaris, P.M., and Mikos, A.G. Modulation of the inflammatory response for enhanced bone tissue regeneration. *Tissue Eng Part B Rev* **14**, 179, 2008.
- Bryers, J.D., Giachelli, C.M., and Ratner, B.D. Engineering biomaterials to integrate and heal: the biocompatibility paradigm shifts. *Biotechnology and bioengineering* **109**, 1898, 2012.
- McNally, A.K., and Anderson, J.M. Macrophage fusion and multinucleated giant cells of inflammation. *Adv Exp Med Biol* **713**, 97, 2011.
- Brown, B.N., Ratner, B.D., Goodman, S.B., Amar, S., and Badylak, S.F. Macrophage polarization: an opportunity for improved outcomes in biomaterials and regenerative medicine. *Biomaterials* **33**, 3792, 2012.
- Jennette, J.C., Falk, R.J., Hu, P., and Xiao, H. Pathogenesis of antineutrophil cytoplasmic autoantibody-associated small-vessel vasculitis. *Annu Rev Pathol* **8**, 139, 2013.
- Burnstock, G. Purinergic signalling: its unpopular beginning, its acceptance and its exciting future. *Bioessays* **34**, 218, 2012.
- Abbraccio, M.P., Burnstock, G., Verkhratsky, A., and Zimmermann, H. Purinergic signalling in the nervous system: an overview. *Trends Neurosci* **32**, 19, 2009.

13. Lohman, A.W., Billaud, M., and Isakson, B.E. Mechanisms of ATP release and signalling in the blood vessel wall. *Cardiovasc Res* **95**, 269, 2012.
14. Anselmi, F., Hernandez, V.H., Crispino, G., Seydel, A., Ortolano, S., Roper, S.D., *et al.* ATP release through connexin hemichannels and gap junction transfer of second messengers propagate Ca^{2+} signals across the inner ear. *Proc Natl Acad Sci U S A* **105**, 18770, 2008.
15. Braunstein, G.M., Roman, R.M., Clancy, J.P., Kudlow, B.A., Taylor, A.L., Shylonsky, V.G., *et al.* Cystic fibrosis transmembrane conductance regulator facilitates ATP release by stimulating a separate ATP release channel for autocrine control of cell volume regulation. *J Biol Chem* **276**, 6621, 2001.
16. Clarke, T.C., Williams, O.J., Martin, P.E., and Evans, W.H. ATP release by cardiac myocytes in a simulated ischaemia model Inhibition by a connexin mimetic and enhancement by an antiarrhythmic peptide. *Eur J Pharmacol* **605**, 9, 2008.
17. Orellana, J.A., Froger, N., Ezan, P., Jiang, J.X., Bennett, M.V., Naus, C.C., *et al.* ATP and glutamate released via astroglial connexin 43 hemichannels mediate neuronal death through activation of pannexin 1 hemichannels. *J Neurochem* **118**, 826, 2011.
18. D'Hondt, C., Ponsaerts, R., Srinivas, S.P., Vereecke, J., and Himpens, B. Thrombin inhibits intercellular calcium wave propagation in corneal endothelial cells by modulation of hemichannels and gap junctions. *Invest Ophthalmol Vis Sci* **48**, 120, 2007.
19. Gomes, P., Srinivas, S.P., Van Driessche, W., Vereecke, J., and Himpens, B. ATP release through connexin hemichannels in corneal endothelial cells. *Invest Ophthalmol Vis Sci* **46**, 1208, 2005.
20. Decrock, E., De Vuyst, E., Vinken, M., Van Moorhem, M., Vranckx, K., Wang, N., *et al.* Connexin 43 hemichannels contribute to the propagation of apoptotic cell death in a rat C6 glioma cell model. *Cell Death Differ* **16**, 151, 2008.
21. Holtzclaw, J.D., Cornelius, R.J., Hatcher, L.I., and Sansom, S.C. Coupled ATP and potassium efflux from intercalated cells. *Am J Physiol Renal Physiol* **300**, F1319, 2011.
22. Lin, J.H., Lou, N., Kang, N., Takano, T., Hu, F., Han, X., *et al.* A central role of connexin 43 in hypoxic preconditioning. *J Neurosci* **28**, 681, 2008.
23. Orellana, J.A., Shoji, K.F., Abudara, V., Ezan, P., Amigou, E., Saez, P.J., *et al.* Amyloid $\{\beta\}$ -induced death in neurons involves glial and neuronal hemichannels. *J Neurosci* **31**, 4962, 2011.
24. Song, D., Liu, X., Liu, R., Yang, L., Zuo, J., and Liu, W. Connexin 43 hemichannel regulates H9c2 cell proliferation by modulating intracellular ATP and $[\text{Ca}^{2+}]$. *Acta Biochim Biophys Sin (Shanghai)* **42**, 472, 2010.
25. Stehberg, J., Moraga-Amaro, R., Salazar, C., Becerra, A., Echeverria, C., Orellana, J.A., *et al.* Release of gliotransmitters through astroglial connexin 43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala. *FASEB J* **26**, 3649, 2012.
26. Di Virgilio, F. Purines, purinergic receptors, and cancer. *Cancer Res* **72**, 5441, 2012.
27. Burnstock, G., and Ulrich, H. Purinergic signaling in embryonic and stem cell development. *Cell Mol Life Sci* **68**, 1369, 2011.
28. Burnstock, G., and Kennedy, C. P2X receptors in health and disease. *Adv Pharmacol* **61**, 333, 2011.
29. Eltzschig, H.K., Sitkovsky, M.V., and Robson, S.C. Purinergic signaling during inflammation. *N Engl J Med* **367**, 2322, 2012.
30. Yuan, D., Wang, Q., Wu, D., Yu, M., Zhang, S., Li, L., *et al.* Monocyte-endothelial adhesion is modulated by Cx43-stimulated ATP release from monocytes. *Biochem Biophys Res Commun* **420**, 536, 2012.
31. McDonald, B., Pittman, K., Menezes, G.B., Hirota, S.A., Slaba, I., Waterhouse, C.C., *et al.* Intravascular danger signals guide neutrophils to sites of sterile inflammation. *Science* **330**, 362, 2010.
32. Davalos, D., Grutzendler, J., Yang, G., Kim, J.V., Zuo, Y., Jung, S., *et al.* ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat Neurosci* **8**, 752, 2005.
33. Chen, Y., Corriden, R., Inoue, Y., Yip, L., Hashiguchi, N., Zinkernagel, A., *et al.* ATP release guides neutrophil chemotaxis via P2Y2 and A3 receptors. *Science* **314**, 1792, 2006.
34. Lemaire, I., Falzoni, S., and Adinolfi, E. Purinergic signaling in giant cell formation. *Front Biosci (Elite Ed)* **4**, 41, 2012.
35. Beis, I., and Newsholme, E.A. The contents of adenine nucleotides, phosphagens and some glycolytic intermediates in resting muscles from vertebrates and invertebrates. *Biochem J* **152**, 23, 1975.
36. Dubyak, G.R., Clifford, E.E., Humphreys, B.D., Kertesz, S.B., and Martin, K.A. Expression of multiple ATP receptor subtypes during the differentiation and inflammatory activation of myeloid leukocytes. *Drug Dev Res* **39**, 269, 1996.
37. Douglas, W.W., and Poisner, A.M. Evidence that the secreting adrenal chromaffin cell releases catecholamines directly from ATP-rich granules. *J Physiol* **183**, 236, 1966.
38. Burnstock, G., and Noradrenaline and ATP: cotransmitters and neuromodulators. *J Physiol Pharmacol* **46**, 365, 1995.
39. Irvin, J.L., and Irvin, E.M. The interaction of quinacrine with adenine nucleotides. *J Biol Chem* **210**, 45, 1954.
40. Alund, M., and Olson, L. Depolarization-induced decreases in fluorescence intensity of gastro-intestinal quinacrine-binding nerves. *Brain Res* **166**, 121, 1979.
41. Sawada, K., Echigo, N., Juge, N., Miyaji, T., Otsuka, M., Omote, H., *et al.* Identification of a vesicular nucleotide transporter. *Proc Natl Acad Sci U S A* **105**, 5683, 2008.
42. Fabbro, A., Skorinkin, A., Grandolfo, M., Nistri, A., and Giniatullin, R. Quantal release of ATP from clusters of PC12 cells. *J Physiol* **560**, 505, 2004.
43. Lazarowski, E.R. Vesicular and conductive mechanisms of nucleotide release. *Purinergic Signal* **8**, 359, 2012.
44. Bodin, P., and Burnstock, G. Purinergic signalling: ATP release. *Neurochem Res* **26**, 959, 2001.
45. Fukuda, Y., and Schuetz, J.D. ABC transporters and their role in nucleoside and nucleotide drug resistance. *Biochem Pharmacol* **83**, 1073, 2012.
46. Pier, G.B. The challenges and promises of new therapies for cystic fibrosis. *J Exp Med* **209**, 1235, 2012.
47. Reisin, I.L., Prat, A.G., Abraham, E.H., Amara, J.F., Gregory, R.J., Ausiello, D.A., *et al.* The cystic fibrosis transmembrane conductance regulator is a dual ATP and chloride channel. *J Biol Chem* **269**, 20584, 1994.
48. Tousson, A., Van Tine, B.A., Naren, A.P., Shaw, G.M., and Schwiebert, L.M. Characterization of CFTR expression and chloride channel activity in human endothelia. *Am J Physiol* **275**, C1555, 1998.
49. Sprague, R.S., Ellsworth, M.L., Stephenson, A.H., Kleinhenz, M.E., and Lonigro, A.J. Deformation-induced

- ATP release from red blood cells requires CFTR activity. *Am J Physiol* **275**, H1726, 1998.
50. Mattoscio, D., Evangelista, V., De Cristofaro, R., Recchiuti, A., Pandolfi, A., Di Silvestre, S., *et al.* Cystic fibrosis transmembrane conductance regulator (CFTR) expression in human platelets: impact on mediators and mechanisms of the inflammatory response. *FASEB J* **24**, 3970, 2010.
 51. Tu, J., Le, G., and Ballard, H.J. Involvement of the cystic fibrosis transmembrane conductance regulator in the acidosis-induced efflux of ATP from rat skeletal muscle. *J Physiol* **588**, 4563, 2010.
 52. Laird, D.W. Life cycle of connexins in health and disease. *Biochem J* **394**, 527, 2006.
 53. Rhett, J.M., Jourdan, J., and Gourdie, R.G. Connexin 43 connexon to gap junction transition is regulated by zonula occludens-1. *Mol Biol Cell* **22**, 1516, 2011.
 54. Rhett, J.M., Ongstad, E.L., Jourdan, J., and Gourdie, R.G. Cx43 Associates with Na(v)1.5 in the Cardiomyocyte Perinexus. *J Membr Biol* **245**, 411, 2012.
 55. Gaietta, G., Deerinck, T.J., Adams, S.R., Bouwer, J., Tour, O., Laird, D.W., *et al.* Multicolor and electron microscopic imaging of connexin trafficking. *Science* **296**, 503, 2002.
 56. Saez, J.C., Berthoud, V.M., Branes, M.C., Martinez, A.D., and Beyer, E.C. Plasma membrane channels formed by connexins: their regulation and functions. *Physiol Rev* **83**, 1359, 2003.
 57. Panchin, Y., Kelmanson, I., Matz, M., Lukyanov, K., Usman, N., and Lukyanov, S. A ubiquitous family of putative gap junction molecules. *Curr Biol* **10**, R473, 2000.
 58. Penuela, S., Bhalla, R., Gong, X.Q., Cowan, K.N., Celetti, S.J., Cowan, B.J., *et al.* Pannexin 1 and pannexin 3 are glycoproteins that exhibit many distinct characteristics from the connexin family of gap junction proteins. *J Cell Sci* **120**, 3772, 2007.
 59. Bao, L., Locovei, S., and Dahl, G. Pannexin membrane channels are mechanosensitive conduits for ATP. *FEBS Lett* **572**, 65, 2004.
 60. Yagi, K., Shinbo, M., Hashizume, M., Shimba, L.S., Kurimura, S., and Miura, Y. ATP diphosphohydrolase is responsible for ecto-ATPase and ecto-ADPase activities in bovine aorta endothelial and smooth muscle cells. *Biochem Biophys Res Commun* **180**, 1200, 1991.
 61. Eltzschig, H.K., Eckle, T., Mager, A., Kuper, N., Karcher, C., Weissmuller, T., *et al.* ATP release from activated neutrophils occurs via connexin 43 and modulates adenosine-dependent endothelial cell function. *Circ Res* **99**, 1100, 2006.
 62. Kansas, G.S., Wood, G.S., and Tedder, T.F. Expression, distribution, and biochemistry of human CD39. Role in activation-associated homotypic adhesion of lymphocytes. *J Immunol* **146**, 2235, 1991.
 63. Thompson, L.F. Crucial role for Ecto-5'-nucleotidase (CD73) in vascular leakage during hypoxia. *J Exp Med* **200**, 1395, 2004.
 64. Hyman, M.C., Petrovic-Djergovic, D., Visovatti, S.H., Liao, H., Yanamadala, S., Bouis, D., *et al.* Self-regulation of inflammatory cell trafficking in mice by the leukocyte surface apyrase CD39. *J Clin Invest* **119**, 1136, 2009.
 65. Moser, G.H., Schrader, J., and Deussen, A. Turnover of adenosine in plasma of human and dog blood. *Am J Physiol* **256**, C799, 1989.
 66. Burnstock, G. Purine and pyrimidine receptors. *Cell Mol Life Sci* **64**, 1471, 2007.
 67. Kaczmarek-Hajek, K., Lorinczi, E., Hausmann, R., and Nicke, A. Molecular and functional properties of P2X receptors—recent progress and persisting challenges. *Purinergic Signal* **8**, 375, 2012.
 68. Solle, M., Labasi, J., Perregaux, D.G., Stam, E., Petrushova, N., Koller, B.H., *et al.* Altered cytokine production in mice lacking P2X(7) receptors. *J Biol Chem* **276**, 125, 2001.
 69. Iyer, S.S., Pulsikens, W.P., Sadler, J.J., Butter, L.M., Teske, G.J., Ulland, T.K., *et al.* Necrotic cells trigger a sterile inflammatory response through the Nlrp3 inflammasome. *Proc Natl Acad Sci U S A* **106**, 20388, 2009.
 70. Ralevic, V., and Burnstock, G. Receptors for purines and pyrimidines. *Pharmacol Rev* **50**, 413, 1998.
 71. Rhett, J.M., Ghatnekar, G.S., Palatinus, J.A., O'Quinn, M., Yost, M.J., and Gourdie, R.G. Novel therapies for scar reduction and regenerative healing of skin wounds. *Trends Biotechnol* **26**, 173, 2008.
 72. Franz, S., Rammelt, S., Scharnweber, D., and Simon, J.C. Immune responses to implants—a review of the implications for the design of immunomodulatory biomaterials. *Biomaterials* **32**, 6692, 2011.
 73. Palatinus, J.A., Rhett, J.M., and Gourdie, R.G. Translational lessons from scarless healing of cutaneous wounds and regenerative repair of the myocardium. *J Mol Cell Cardiol* **48**, 550, 2010.
 74. Dovi, J.V., He, L.K., and DiPietro, L.A. Accelerated wound closure in neutrophil-depleted mice. *J Leukoc Biol* **73**, 448, 2003.
 75. Degen, K.E., and Gourdie, R.G. Embryonic wound healing: a primer for engineering novel therapies for tissue repair. *Birth Defects Res C Embryo Today* **96**, 258, 2012.
 76. Ghatnekar, G.S., O'Quinn, M.P., Jourdan, L.J., Gurjarpadhye, A.A., Draughn, R.L., and Gourdie, R.G. Connexin43 carboxyl-terminal peptides reduce scar progenitor and promote regenerative healing following skin wounding. *Regen Med* **4**, 205, 2009.
 77. O'Quinn, M.P., Palatinus, J.A., Harris, B.S., Hewett, K.W., and Gourdie, R.G. A Peptide mimetic of the connexin43 carboxyl terminus reduces gap junction remodeling and induced arrhythmia following ventricular injury. *Circ Res* **108**, 704, 2011.
 78. Soder, B.L., Propst, J.T., Brooks, T.M., Goodwin, R.L., Friedman, H.I., Yost, M.J., *et al.* The connexin43 carboxyl-terminal peptide ACT1 modulates the biological response to silicone implants. *Plast Reconstr Surg* **123**, 1440, 2009.
 79. Monroe, D.M., Hoffman, M., and Roberts, H.R. Platelets and thrombin generation. *Arterioscler Thromb Vasc Biol* **22**, 1381, 2002.
 80. Singer, A.J., and Clark, R.A. Cutaneous wound healing. *N Engl J Med* **341**, 738, 1999.
 81. Cote, Y.P., Filep, J.G., Battistini, B., Gauvreau, J., Sirois, P., and Beaudoin, A.R. Characterization of ATP-diphosphohydrolase activities in the intima and media of the bovine aorta: evidence for a regulatory role in platelet activation *in vitro*. *Biochim Biophys Acta* **1139**, 133, 1992.
 82. Komoszynski, M., and Wojtczak, A. Apyrases (ATP diphosphohydrolases, EC 3.6.1.5): function and relationship to ATPases. *Biochim Biophys Acta* **1310**, 233, 1996.

83. Woulfe, D., Yang, J., and Brass, L. ADP and platelets: the end of the beginning. *J Clin Invest* **107**, 1503, 2001.
84. Clark, R. Wound repair overview and general considerations. In: Clark, R., ed. *The Molecular and Cellular Biology of Wound Repair*. New York: Plenum Press, 1996, pp. 3–50.
85. Kahner, B.N., Shankar, H., Murugappan, S., Prasad, G.L., and Kunapuli, S.P. Nucleotide receptor signaling in platelets. *J Thromb Haemost* **4**, 2317, 2006.
86. Burnstock, G., Knight, G.E., and Greig, A.V. Purinergic signaling in healthy and diseased skin. *J Invest Dermatol* **132**, 526, 2012.
87. Godecke, S., Roderigo, C., Rose, C.R., Rauch, B.H., Godecke, A., and Schrader, J. Thrombin-induced ATP release from human umbilical vein endothelial cells. *Am J Physiol Cell Physiol* **302**, C915, 2012.
88. Inkinen, K. *Connective Tissue Formation in Wound Healing* Department of Biosciences, University of Helsinki, Helsinki, [Academic Dissertation], Juhani Ahonen, Department of Surgery, Helsinki University Central Hospital, 2003. ISBN952-10-1313-3
89. Hubner, G., Brauchle, M., Smola, H., Madlener, M., Fassler, R., and Werner, S. Differential regulation of pro-inflammatory cytokines during wound healing in normal and glucocorticoid-treated mice. *Cytokine* **8**, 548, 1996.
90. Tonnesen, M.G., Worthen, G.S., and Johnston, R.B.J. Neutrophil emigration, activation, and tissue damage. In: Clark, R.A.F., and Henson, P.M., eds. *The Molecular and Cellular Biology of Wound Repair*. New York: Plenum Press, 1988, pp. 149–183.
91. Chen, Y., Yao, Y., Sumi, Y., Li, A., To, U.K., Elkhail, A., *et al.* Purinergic signaling: a fundamental mechanism in neutrophil activation. *Sci Signal* **3**, ra45, 2010.
92. Barletta, K.E., Ley, K., and Mehrad, B. Regulation of neutrophil function by adenosine. *Arterioscler Thromb Vasc Biol* **32**, 856, 2012.
93. Zimmermann, H., and Braun, N. Extracellular metabolism of nucleotides in the nervous system. *J Auton Pharmacol* **16**, 397, 1996.
94. Ponsaerts, R., De Vuyst, E., Retamal, M., D'Hondt, C., Vermeire, D., Wang, N., *et al.* Intramolecular loop/tail interactions are essential for connexin 43-hemichannel activity. *FASEB J* **24**, 4378, 2010.
95. Bodin, P., and Burnstock, G. Increased release of ATP from endothelial cells during acute inflammation. *Inflamm Res* **47**, 351, 1998.
96. Wan, J., Ristenpart, W.D., and Stone, H.A. Dynamics of shear-induced ATP release from red blood cells. *Proc Natl Acad Sci U S A* **105**, 16432, 2008.
97. Morais, J.M., Papadimitrakopoulos, F., and Burgess, D.J. Biomaterials/tissue interactions: possible solutions to overcome foreign body response. *AAPS J* **12**, 188, 2010.
98. Robson, S.C., Candinas, D., Hancock, W.W., Wrighton, C., Winkler, H., and Bach, F.H. Role of endothelial cells in transplantation. *Int Arch Allergy Immunol* **106**, 305, 1995.
99. Dormond, O., Dufour, M., Seto, T., Bruneau, S., and Briscoe, D.M. Targeting the intragraft microenvironment and the development of chronic allograft rejection. *Hum Immunol* **73**, 1261, 2012.
100. Luttikhuisen, D.T., Harmsen, M.C., de Leij, L.F., and van Luyn, M.J. Expression of P2 receptors at sites of chronic inflammation. *Cell Tissue Res* **317**, 289, 2004.
101. Varoni, E., Tschon, M., Palazzo, B., Nitti, P., Martini, L., and Rimondini, L. Agarose gel as biomaterial or scaffold for implantation surgery: characterization, histological and histomorphometric study on soft tissue response. *Connect Tissue Res* **53**, 548, 2012.
102. Gilmartin, D.J., Alexaline, M.M., Thrasyvoulou, C., Phillips, A.R., Jayasinghe, S.N., and Becker, D.L. Integration of scaffolds into full-thickness skin wounds: the connexin response. *Adv Healthcare Mater* **2**, 1151, 2013.
103. Waldeck, H., Wang, X., Joyce, E., and Kao, W.J. Active leukocyte detachment and apoptosis/necrosis on PEG hydrogels and the implication in the host inflammatory response. *Biomaterials* **33**, 29, 2012.
104. Spargo, B.J., Rudolph, A.S., and Rollwagen, F.M. Recruitment of tissue resident cells to hydrogel composites: *in vivo* response to implant materials. *Biomaterials* **15**, 853, 1994.
105. Pol, B.J., van Wachem, P.B., van Luyn, M.J., van der Does, L., and Bantjes, A. *In vivo* testing of crosslinked polyethers. I. Tissue reactions and biodegradation. *J Biomed Mater Res* **32**, 307, 1996.
106. Fann, S.A., Terracio, L., Yan, W., Franchini, J.L., and Yost, M.J. A model of tissue-engineered ventral hernia repair. *J Invest Surg* **19**, 193, 2006.
107. Don, C.W., and Murry, C.E. Improving survival and efficacy of pluripotent stem cell-derived cardiac grafts. *J Cell Mol Med* **17**, 1355, 2013.
108. Laflamme, M.A., and Murry, C.E. Heart regeneration. *Nature* **473**, 326, 2011.
109. Laflamme, M.A., and Murry, C.E. Regenerating the heart. *Nat Biotechnol* **23**, 845, 2005.
110. Terrovitis, J., Stuber, M., Youssef, A., Preece, S., Leppo, M., Kizana, E., *et al.* Magnetic resonance imaging overestimates ferumoxide-labeled stem cell survival after transplantation in the heart. *Circulation* **117**, 1555, 2008.
111. Mayfield, A.E., Tilokee, E.L., Latham, N., McNeill, B., Lam, B.K., Ruel, M., *et al.* The effect of encapsulation of cardiac stem cells within matrix-enriched hydrogel capsules on cell survival, post-ischemic cell retention and cardiac function. *Biomaterials* **35**, 133, 2013.
112. Friedman, H.I., Friedman, A.C., and Carson, K. The fate of the fibrous capsule after saline implant removal. *Ann Plast Surg* **46**, 215, 2001.
113. Bender, J.W., Friedman, H.I., Giurgiutiu, V., Watson, C., Fitzmaurice, M., and Yost, M.L. The use of biomedical sensors to monitor capsule formation around soft tissue implants. *Ann Plast Surg* **56**, 72, 2006.
114. Komoszynski, M.A. Comparative studies on animal and plant apyrases (ATP diphosphohydrolase EC 3.6.1.5) with application of immunological techniques and various ATPase inhibitors. *Comp Biochem Physiol B Biochem Mol Biol* **113**, 581, 1996.
115. Takahashi, T., Kimura, Y., Niwa, K., Ohmiya, Y., Fujimura, T., Yamasaki, K., *et al.* *In vivo* imaging demonstrates ATP release from murine keratinocytes and its involvement in cutaneous inflammation after tape-stripping. *J Invest Dermatol* **133**, 2407, 2013.
116. Amaral, S.S., Oliveira, A.G., Marques, P.E., Quintao, J.L., Pires, D.A., Resende, R.R., *et al.* Altered responsiveness to extracellular ATP enhances acetaminophen hepatotoxicity. *Cell Commun Signal* **11**, 10, 2013.
117. Reutershan, J., Vollmer, I., Stark, S., Wagner, R., Ngamsri, K.C., and Eltzschig, H.K. Adenosine and inflammation: CD39 and CD73 are critical mediators in LPS-induced PMN trafficking into the lungs. *FASEB J* **23**, 473, 2009.
118. Tsai, T.L., Chang, S.Y., Ho, C.Y., and Kou, Y.R. Role of ATP in the ROS-mediated laryngeal airway

- hyperreactivity induced by laryngeal acid-pepsin insult in anesthetized rats. *J Appl Physiol* **106**, 1584, 2009.
119. Idzko, M., Hammad, H., van Nimwegen, M., Kool, M., Willart, M.A., Muskens, F., *et al.* Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nat Med* **13**, 913, 2007.
120. Eckle, T., Fullbier, L., Wehrmann, M., Khoury, J., Mittelbronn, M., Ibla, J., *et al.* Identification of ectonucleotidases CD39 and CD73 in innate protection during acute lung injury. *J Immunol* **178**, 8127, 2007.
121. Koyamada, N., Miyatake, T., Candinas, D., Hechenleitner, P., Siegel, J., Hancock, W.W., *et al.* Apyrase administration prolongs discordant xenograft survival. *Transplantation* **62**, 1739, 1996.

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