

REVIEW

Electrospun Nanofibers as Support for the Healing of Intestinal Anastomoses

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Summary

The breakdown of intestinal anastomosis is a serious postsurgical complication. The worst complication is anastomotic leakage, resulting in contaminated peritoneal cavity, sepsis, multi-organ failure and even death. In problematic locations like the rectum, the leakage rate has not yet fallen below 10 %. Such a life-threatening condition is the result of impaired healing in the anastomotic wound. It is still vital to find innovative strategies and techniques in order to support regeneration of the anastomotic wound. This paper reviews the surgical techniques and biomaterials used, tested or published. Electrospun nanofibers are introduced as a novel and potential material in gastrointestinal surgery. Nanofibers possess several, unique, physical and chemical properties, that may effectively stimulate cell proliferation and collagen production; a key requirement for the healed intestinal wound.

Key words

Regeneration • Colorectal anastomosis • Surgical techniques • Biomaterials • Artificial ECM

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Introduction

An anastomosis is a surgical connection between two hollow body structures that transport fluids, such as blood vessels or ducts of the urinary and gastrointestinal tract (GIT). In gastrointestinal surgery, anastomoses are the most common surgical procedures. However, not all parts of the GIT have the same healing rate as anastomoses. The most problematic parts are mainly the esophagus, the large intestine (colon) and the rectum. Therefore, colorectal anastomoses, the connections between the large bowel and the rectum, have a relatively high rate of morbidity and mortality in comparison to the small intestine. Numerous studies have shown a decreased healing rate in this problematic location of the GIT (Buchs *et al.* 2008, Boccola *et al.* 2011, Krarup *et al.* 2012).

This study gives a brief overview of the surgical techniques and biomaterials used to support healing in intestinal anastomoses. Special attention is paid to electrospun nanofibers, versatile biomaterials that could directly stimulate cells and enhance the regeneration potential in problematic colorectal anastomoses.

The construction of intestinal anastomosis

A surgical technique has paramount importance

for the healing of intestinal anastomosis in the GIT. The wall of the GIT consists of four layers: mucosa, submucosa, muscular layer and serosa. Only the esophagus and the lower third of the rectum lack the serosa layer. During an intestinal resection, cutting off part of an intestine, all four layers are completely removed, breaking the continuity of the GIT. Continuity of the GIT is restored after anastomosis construction, using an end-to-end, end-to-side or a side-to-side technique. Anastomosis can be sewn by hand or done mechanically, using a stapler. Mechanical construction is used after rectum resection that is located deep in the pelvic cavity. In this location with such limited access, sewing the anastomosis by hand is often very difficult or impossible. Therefore, an intraluminal circular stapler is transanally inserted into the rectum and parts of the intestine are connected and fixed by titanium staples (Joyce *et al.* 2002).

Hand sewing enables a variety of suturing techniques. Sutures can be made with a single strand of suture material, continuous suture, or by a series of individual stitches that are not connected, interrupted suture (Sliker *et al.* 2013). Additionally, anastomosis can be sewn in a single layer extramucosal or in a double layer. Due to the double suture, it was believed that the double layered anastomosis is safer than the single layered one. However, Kar *et al.* 2017 concluded from a comparative statistical study, that the single layered extramucosal technique is equally as safe as the double layered one. Sutures in the single layered extramucosal anastomosis stitch the serosa and submucosa. The latter layer contains interwoven fibers of collagen (type I, III and V) and elastic fibers; the layer is responsible for the majority of tensile strength in intact GIT. Consequently, submucosa is suitable for anchoring the suture in single layered anastomosis and for a more precise layer apposition. A double layered anastomosis consists of an inner suture stitching all layers, and an outer suture through the serosa and muscular layer. Extra sutures do more harm to submucosal neural and vascular plexus that may negatively affect sufficient nutrition and oxygen, key factors for cell proliferation and collagen production and healing of the intestinal wound.

The healing of intestinal anastomoses

The healing of intestinal anastomosis is a complex process divided into three overlapping stages (phases): inflammatory stage (lag phase, days 0-4 after

surgery), proliferation stage (phase of fibroplasia, days 3-14) and the stage of tissue reorganization (maturation phase, day 10 onwards) (Thornton and Barbul 1997, Ashkanani and Krukowski 2002). Immediately after the gut resection, the gut incision initially triggers hemostatic vasoconstriction, followed by secondary vasodilatation (Thornton and Barbul 1997). The widening of blood vessels and the increasing of their permeability causes swelling to both ends of the incised gut. Vasodilatation also helps leukocytes to migrate into the wound site. Leukocytes, granulocytes and macrophages clear the wound of necrotic tissue, debris and pathogens. Leukocytes release signal molecules such as cytokines that, along with hypoxia, stimulate the formation of neovascularization. New vessels in the wound carry blood with nutrition and oxygen, essential factors for collagen synthesis and the formation of granulation tissue. The granulation tissue serves as a scaffold for the migration of epithelia cells from mucosa. The new mucosal covering, seals the wound from the luminal side and protects to allow intestinal content to enter into the wound. The appearance of granulation tissue is a sign of the next stage of healing. During the proliferation stage, cells proliferate and massively produce collagen. In contrast to the skin wound, smooth muscle cells produce collagen as well as fibroblast in the intestinal wound. The immature collagen and the intestinal wound are remodeled during the final stage of tissue reorganization.

Intensity, timing and the duration of the stages, differs for different parts of the GIT. For example, in rabbits some differences were observed between the anastomoses healing of ileum, the final part of the small intestine and colon, the large intestine (Hesp *et al.* 1985). Seven days after surgery, granulocytes and necrosis were still abundantly present in the colonic anastomoses, but absent in the ileal ones. Additionally, in the colonic anastomoses, colonic mucosae were poorly repaired in contrast to completely healed ileal mucosae in the majority of cases examined. Overall, the healing rate of colonic anastomoses was significantly lower than in ileal anastomoses. Authors ruled out a different blood flow in the ileum and colon. They expressed the longer presence of granulocytes in the colon to be a more likely reason. Granulocytes produce collagenases that digest collagen (Lazarus *et al.* 1968). This group of enzymes plays an important role during the wound clearing of debris and during the reorganization of collagen fibrils in the granular tissue and the wound. In colonic anastomoses, mainly in the suture-holding zone, a striking collagen loss

(29 % drop compared with adjacent micro-areas) and a higher concentration of collagenases (MMP-8 and MMP-9) were histochemically detected (Agren *et al.* 2006). However, a high concentration of collagenases may hinder the formation of granulation tissue, a key intermediate of intestine wound healing. A lower amount of collagen weakens the tensile strength of anastomosis and may lead to serious postsurgical complications.

Anastomotic leakage

One of the most serious complications in gastrointestinal surgery is anastomotic dehiscence; a breakdown of anastomosis leading to anastomotic leak. Anastomotic leak is an anastomosis defect through which intestinal lumen communicates with the peritoneal cavity (Fig. 1). In the most serious of cases, an anastomotic leak may be a potentially life-threatening condition. The luminal content of the intestines contaminates the peritoneal cavity leading to stercoral peritonitis, sepsis, subsequent multi-organ failure and death. To save the life of the patient, anastomosis has to be re-operated to remove previous anastomosis and to construct a new one (Thornton *et al.* 2011). After re-operation, the intestinal content is diverted from anastomosis to a stoma, an artificial opening on the body surface.

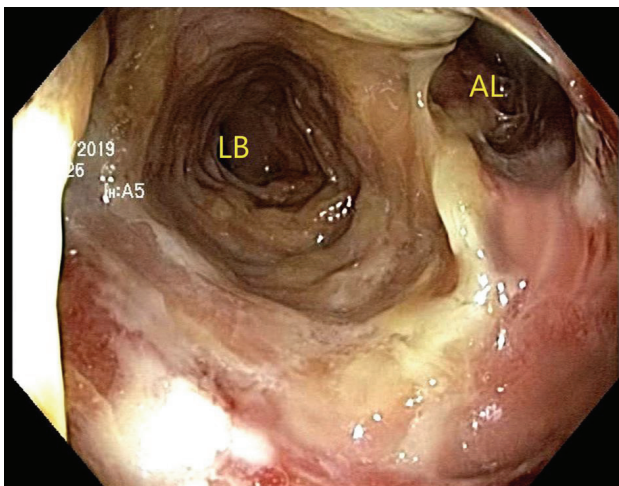


Fig. 1. Endoscopic view of the large bowel (LB) with an anastomotic leak (AL).

The leakage rate in colorectal anastomoses is still relative high, around 10 % (Chambers and Mortensen 2004, Olsen *et al.* 2019). There are several factors that increase the risk of anastomotic leakage (Kato *et al.* 2011, Volk *et al.* 2011, Jannash *et al.* 2015); the most

well-known being age, male gender, followed by associated diseases and medication (e.g. cancer, immunosuppressive and non-steroidal drugs, chemotherapeutics), ASA III and the higher classification of physical status (by American Society of Anesthesiologists), nutrition, obesity, smoking, alcohol consumption, sepsis and ileus. Absent or changed intestinal bacterial microflora may negatively affect the healing of the mucosa layer in the intestinal anastomoses (Shogan *et al.* 2013).

The main goal is to prevent or minimize the risk of postsurgical complications such as anastomotic leakage. One approach is adequate perioperative care – type of anesthesia and analgesia, antibiotic prophylaxis, protective stoma and appropriate techniques of anastomosis that support healing (Daams *et al.* 2013). For example, if intestinal layers are directly apposed, mucosal sealing can be completed in three days following surgery. However, in the case of mucosal eversion and inversion, sealing formation is likely to be delayed due to a longer path for migrating epithelial mucosal cells (Thornton and Barbul 1997). In addition, a precise layer apposition is a key factor for serosa healing.

Biomaterials supporting the healing of intestinal anastomosis

Another approach to prevent or minimize the risk of anastomotic leakage is using novel biomaterials that are able to boost the natural regenerative capabilities of intestinal tissues. The first type of biomaterials provides mechanical support to anastomosis. Perfect apposition of all intestinal layers is vital for successfully healed intestinal wounds. The simplest and most used versions of mechanical support are titanium staples and permanent or biodegradable sutures. Novel sutures innovated by a coating of a collagenases inhibitor, doxycycline, showed positive results in rat colonic anastomosis (Pasternak *et al.* 2008). In comparison to the small intestine, the colon and rectum exhibit a slower healing rate that is often linked with an increased concentration of collagenases. The breaking strength of the healed anastomosis increased by 17 % compared to the non-coated sutures. Aysan *et al.* 2008 used polypropylene mesh, normally used in hernia surgery, for the external covering of anastomosis after resection of the proximal colon in rabbits. Polypropylene mesh, as an external mechanical buttress, was proposed to reduce the intraluminal pressure caused by intestinal movements.

As the results showed, polypropylene mesh mechanically reinforced the anastomotic site. Mechanical tests were performed by filling up the colon with water and exerting bursting pressure. To burst anastomosis 10 days after surgery, covered anastomoses needed more than twice as high bursting pressure. However, polypropylene is not a biodegradable polymer and mesh and non-absorbable sutures may negatively interact with the colon. Therefore, it is better to use meshes made of biodegradable synthetic polymers such as polyglycolic acid (PGA). Nevertheless, biodegradable synthetic polymers such as PGA or poly- ϵ -caprolactone (PCL) degrade in weeks or months (Castilla-Cortázar *et al.* 2012) in comparison with several days, an average healing time for problem-free intestinal anastomoses (Thornton and Barbul 1997). The biodegradability rate of material prepared from synthetic polymers also depends on the shape and thickness of a material. Accordingly, there is a tendency to prepare an extremely thin external covering with a thickness of several tens-of-nanometers. As such, nanomembranes very accurately cover the external surface contours of anastomosis and work as a seal. A PLLA nanomembrane without sutures was successfully tested on mouse gastric incisions (Okamura *et al.* 2009). No studies testing nanomembranes on bigger animal models such as pigs have yet been published.

A more progressive attitude for the healing of intestinal anastomoses, is using a material that directly stimulates cells to proliferate and differentiate into functional tissues. The key is to prepare an appropriate, artificial environment that structurally and functionally mimics a natural one. In intestinal tissues, cells like fibroblasts and smooth muscle cells naturally live in a network of thin fibers of extracellular matrix (ECM), mainly composed of collagens. The ECM has many essential functions for cells. The ECM provides mechanical protection for cells and serves as a surface for cells to attach. It is well known that anchorage dependent cells like fibroblasts, without sufficient attachment and adhesion to an appropriate surface, are not able to proliferate and differentiate. In the extreme, some types of cells undergo cell death without cell adhesion to a substrate (Straface *et al.* 1999). Cell adhesion is mediated by sophisticated protein machineries that transduce mechanical signals into intracellular chemical ones, change cell shape and trigger gene expression affecting cell behavior like cell division or differentiation (Choquet *et al.* 1997, Schmidt *et al.* 1998, Chien *et al.* 1998). Differentiating fibroblasts produce collagen and as

a positive feedback, collagen, the main protein of the ECM, provides excellent cell adhesion, proliferation, differentiation and thus its own production that may result in mechanically stronger anastomosis.

Collagen, as a natural component of tissues, is often used for the preparation of biomaterials. Decellularized collagen patches obtained from porcine skin, were successfully used as an external covering of a dog small intestine (Hori *et al.* 2001). Prior to application on the serosal surface, the collagen patches were soaked with blood. Intestinal and skin collagens have a different chemical composition. Intestine has collagen type I, III and V, while skin collagen is type I and II. Despite different collagens, skin collagen patches were integrated into submucosa. In the study, the blood soaking of collagen patches could have a substantial healing effect for the anastomotic wound. In fact, platelets in blood are a natural source of molecular signals such as growth factors that stimulate cells to proliferate. The positive effect of platelets confirmed another study on rat colonic anastomosis (Yol *et al.* 2007). A group of colon anastomosis with typically applied PRP (platelets rich plasma) showed significantly higher bursting pressure and higher collagen production than the control groups. An innovative approach, sealing collagen patches on colonic anastomosis by fibrin glue, showed no differences in healing or infection (Nordentoft *et al.* 2007). However, the positive effect on the healing of intestinal anastomosis with fibrin glue only, is a subject of discussion. A meta-analysis from 2015 concluded that fibrin glue had no positive effects on the healing of gastrointestinal anastomoses (Nordentoft *et al.* 2015). The effect of fibrin glue is rather as a mechanical support. Some studies with fibrin glue often report problems with inflammation accompanied by a higher amount of granulocytes in the anastomotic wound (e.g., Ozel *et al.* 2006). Fibrin, as a product of blood clotting, occurs naturally in the wound. In cutaneous wounds, fibrin and mainly its metabolites as well as enzymatic active thrombin attract inflammatory cells, granulocytes and monocytes, from the blood (Richardson *et al.* 1976, Gross *et al.* 1997, Bar-Shavit *et al.* 1990). Granulocytes enter the wound site and clear the wound of fibrin by digesting it. Granulocytes greatly participate in inflammation management in the wound. After wound entering, monocytes mature into macrophages. A higher amount of granulocytes macrophages leads to increased collagenase production that has a negative impact on collagen production and healing. Collagenases reduce the

healing rate of anastomotic wounds. A worse effect than fibrin glue on the healing of intestinal anastomoses is from cyanoacrylate glue, a synthetic glue used in surgery (Bae *et al.* 2010). The author noticed decreased collagen content in anastomotic wound and intensive wound inflammation. Polymeric fibers of cyanoacrylate were most likely recognized as a foreign object that triggered an immunity reaction. Such a material cannot be an artificial ECM.

Electrospun nanofibers

It is a considerable challenge to prepare material with the structure and function of artificial ECM. Such a material should meet a lot of requirements. The surface properties of a material should allow appropriate cell adhesion. Cells generally prefer hydrophilic surfaces (Tamada and Ikada 1993, Lee *et al.* 1998). Excessively hydrophobic surfaces repulse water molecules that are essential for the functional conformation of proteins mediating cell attachment (Mrksich and Whitesides 1996). This material should be fabricated from polymers that are biocompatible and fully biodegradable, in an ideal case within one week following the application on an anastomosis, the material should disappear. The material should not enhance an immune response. This material should be firm and resilient but also pliable and elastic in respect to the nature of the gastrointestinal tract. The shape and the size of a material should be stable, shortening of a material could lead to a stricture or closure of the suture. Additionally, this material should be porous and pores should be interconnected for efficient transport of nutrients, waste metabolites and signal molecules between cells in the suture. To avoid infection of the peritoneal cavity, pores of a material should be smaller than the average size of a bacterial cell, approximately below one micrometer. The thickness of collagen I and collagen III fibers varies from hundreds of nanometers to several micrometers (0.2 - 4 μm in rat intestine, Orberg *et al.* 1982). Therefore, this material should consist of fibers that structurally and functionally resemble a network of collagen fibers. To prepare material made of such fine fibers with the mentioned properties, a nanotechnology is needed.

Electrospinning seems to be such a nanotechnology. Electrospinning is a nanotechnology for preparing fine fibers with a diameter ranging from 1.6 nm to several micrometers (Huang *et al.* 2006). Electrospinning uses electrical forces that deform

a droplet of polymeric solution into a structure called Taylor cone (Ramakrishna 2005, Reneker and Yarin 2008). In an increasing electrical field, Taylor cone prolongs to a thinner structure termed a jet. Prolonging and thinning continues and the jet is transformed into a fine fiber of polymeric solution. The thinner the fiber is, the bigger the surface area-to-volume ratio is and the faster the solvent evaporates from the fiber that causes solidifying of the fiber. The interface solid-liquid is mechanically unstable and in this area the fiber breaks. Free fiber is attracted to an opposite charged metal plate, a collector. The deposition of many nanofibers on a collector lead to the emergence of a macroscopically nanofibrous material that we are able to manipulate.

Nanofibers have a lot of unique properties that could be useful for application on colorectal anastomoses. Fine fibers structurally mimic the collagen network. Material composed of such fine fibers has a high surface area to volume ratio (Gibson *et al.* 2001). It means that a relatively large surface area of the material is exposed to body liquids. This causes better access of enzymes to nanofibers and faster biodegradation. A high surface area to volume ratio is also favorable for efficient drug delivery. In fact, during electrospinning, it is possible to encase a drug to nanofibers. As the structure of nanofibers is degraded over time, an encased drug is released into the environment. In respect to the nature of gastrointestinal anastomoses and the possible infection of the peritoneal cavity, the encasement of antibiotics could be efficient in reducing postsurgical complications. Also the encasement of ascorbic acid, as a key factor in collagen synthesis, could hasten the healing of anastomoses. For effective drug delivery, a nanofibrous material should have high porosity with interconnected pores. Nanofibers are very often mostly interwoven into each other, surrounded with a lot of free space, making nanofibrous material highly porous and the pores communicate with each other. Ziabari *et al.* 2008 showed that pore diameter and the porosity of nanofibers are controllable. Generally, the pore diameter of nanofibers is below one micrometer (Fig. 2). Therefore, nanofibers are impenetrable for bacteria living in gastrointestinal lumen (Bjorge *et al.* 2009).

Electrospinning is a versatile technique for preparing nanofibers from more than 100 polymers, including some polymers approved for human use (Rošic *et al.* 2012). Polymeric chains of water soluble polymers like polyvinyl alcohol (PVA) are possible to crosslink and make nanofibers stable in a wet environment (Ding

et al. 2002). Moreover, the intensity of crosslinking takes half the time of dissolution and enables the tailoring of material to the appropriate degradation rate or (and) the release of encased drugs from nanofibers into a suture (Zhang *et al.* 2016).

When a static metal plate is replaced by a high speed (500 rpm and more) rotating metal drum, the

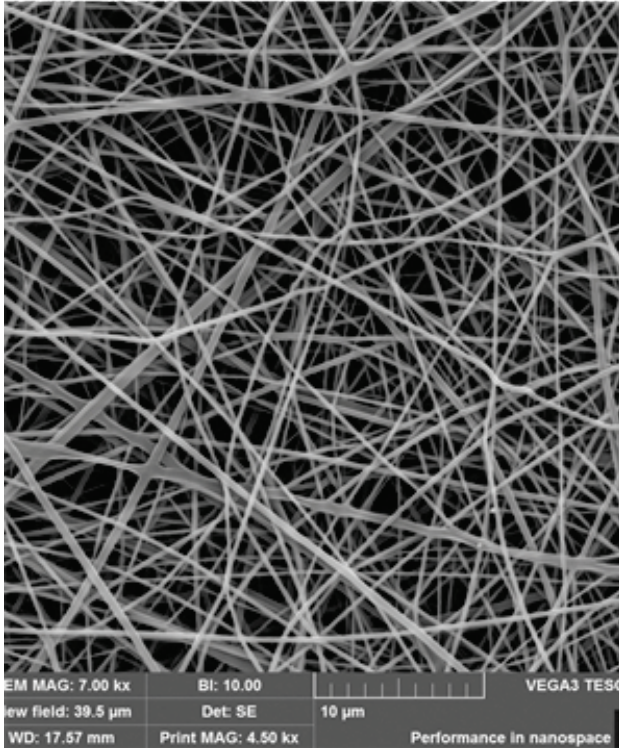


Fig. 2. Polyvinyl alcohol nanofibers prepared by the electrospinning technique (magnification 7 000x).

nanofiber will be mainly oriented perpendicular to an axis of rotation (Matthews *et al.* 2002). As a result, material made of uniaxial or parallel aligned nanofibers is mechanically more resistant in one direction. When parallel aligned nanofibers are then turned 90° on a rotating collector, a new layer of parallel aligned nanofibers are obtained, perpendicular to the previous one (Li *et al.* 2004). Such layer-by-layer stacked material has significantly more mechanical firmness than nanofibrous material with randomly aligned nanofibers. Using thinner material speeds up its degradation. The nanofiber arrangement has considerably impacted on the ability of cells to migrate. Normally, the effectiveness of healing decreases from periphery to the centrum of suture due to a shortage of nutrients, oxygen and proliferating cells. The parallel alignment of nanofibers facilitates cell migration. In vitro tests showed that for example, human

glioma cells migrated approximately five times faster on parallel aligned nanofibers than on random ones (Johnson *et al.* 2009). Fibroblasts migrated faster on 8 µm microfibers than on films (Quin *et al.* 2015) Moreover, Tian *et al.* 2008 have discovered that parallel aligned fibers are more suitable for fibroblasts in terms of proliferation, migration and differentiation, than randomly aligned ones. Parallel arranged nanofibers applied perpendicular to the suture of an anastomosis, could make healing of the anastomosis faster and more effective.

Conclusion

The colorectal anastomoses are still a problematic issue in gastrointestinal surgery. In comparison to another intestinal anastomoses, the colorectal anastomoses heal at a slower rate which can lead to postsurgical complications. A life-threatening complication is a dehiscence, often resulting into an anastomotic leakage. The leakage rate in the colorectal anastomosis is around 10 % that is relative high in comparison to another intestinal anastomoses (Chambers and Mortensen 2004, Olsen *et al.* 2019). The most effective way to avoid anastomotic leakage is perfectly performed surgery with precise apposition of all four intestinal layers and appropriate perioperative care. Despite fulfilling the aforementioned requirements, there are patients with factors (e.g. age, associated diseases, medication) that increase risk of anastomotic leakage.

In order to decrease the risk of leakage, some biomaterials are developed, tested and used in the surgical praxis. Sutures, staples, meshes and membranes are designed for the biomechanical support of anastomotic wounds and for reduction of the intraluminal pressure caused by intestinal movements. In rabbits, sutures coated with collagenases inhibitor, doxycycline, decreased concentration of collagenases and an increased concentration of collagen, are key factor for wound healing and the biomechanical strength of the healed wound (Pasternak *et al.* 2008).

A more promising approach involves using a material that stimulates cells to proliferate and differentiate to the intestinal tissues. Fibrin glue, a material used in humans, provide rather biomechanical support than stimulation of fibroblasts and smooth muscle cells (Nordentoft *et al.* 2015). However, fibrin glue stimulates inflammatory cells and leads to an increase of collagenases concentration and risk of

dehiscence. Patches made of synthetic or natural polymers like collagen serve as a scaffold for cells in the anastomotic wound (Hori *et al.* 2001).

Nanofibers prepared by electrospinning are a unique material and with their structure and function resembles the extracellular matrix found in tissues. With high free surface energy, they are perfect for cell attachment, a requirement for cell proliferation and differentiation. The highly porous material with interconnected pores enables effective diffusion of waste metabolites and nutrients. Electrospinning is a versatile technique that allows preparation of parallel nanofibers, which accelerate cell migration from the periphery to the centrum of the wound. Nanofibers are a potential material

to support the healing of colorectal or even other types of anastomoses in surgery.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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