



Review

Orthotopic grafting of decellularized human nipple: Setting the stage and putative mechanism of healing



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ABSTRACT

A complex skin structure (such as a nipple) can be successfully decellularized under conditions that prevent extracellular matrix crosslinking or undue matrix degradation [1]. This treatment removes cellular antigens, thus mitigating immunorejection concerns and enabling allogeneic transplantation for nipple reconstruction after mastectomy. Non-human primate studies have shown that host-mediated re-vascularization and re-epithelization of the decellularized nipples occurs within six weeks and nipple projection is maintained over the same timeframe [1]. The mechanisms by which a decellularized graft located on the surface of the body heals are incompletely understood, but are likely to follow a similar path to decellularized allografts that are implanted within the body, with some modifications. The following is a description of probable temporal events leading to healing under this circumstance.

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1. Nipple reconstruction

At some relevant timepoint after mastectomy and breast reconstruction, a woman will elect to have nipple reconstruction. After a review of all possible techniques, including tattooing, local flap rotation, autologous full thickness skin grafting from labia, and allogeneic or xenogeneic implants, it becomes clear that all are flawed in some way that prevents the accurate reproduction of a nipple's 3D structure, pliancy, and color. A proposed new solution is to take advantage of the extensive evidence that a decellularized

allogeneic or xenogeneic graft, an acellular extracellular matrix scaffold, can serve as the nidus for a healing event when implanted in a human [2]. Although such scaffolds have long been derived from dermis, small intestinal submucosa, bladder and other organs, recent work has shown that deceased human donor nipples can be successfully decellularized and, when implanted on the surface of the body in a non-human primate model, exhibit complete healing within six weeks [1]. Planned clinical studies will bring this technology to humans, where longed for accurate and complete nipple restoration can be evaluated.

When a woman elects nipple reconstruction with a decellularized human nipple, it is anticipated the following sequence of surgical events will occur. First, she will undergo presurgical evaluation to ensure that her reconstructed breast(s) have healed

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properly and bear no residual inflammation. Prior to surgery, she will be consulted regarding the relative size and placement of her new nipple(s). Markings will be made to denote these dimensions and positions and standard preparations for surgery will be made. Whether under local or general anesthesia, the breast skin will be prepped with an antiseptic, and prophylactic antibiotics will likely be administered. The surgeon will then remove the epithelial layer of the skin in the marked nipple location(s) on the breast(s), exposing the vascularized dermis, below. Punctate bleeding will likely require control by pressure or electrocautery to create a blood-free recipient field. The decellularized nipple(s) and surrounding areola will then be cut to the desired shape and sewn onto the breast(s) with either interrupted or continuous sutures. Wound dressings will be placed to encompass the nipple(s) but not compress the projecting nipple, itself. It is clear from the outset that this form of decellularized scaffold placement on the human body differs from other scaffold implantations in that it faces a vascularized interface only on one side. So, what is the likely pathway to its healing response?

2. Healing of intact and acellular grafts

There will likely be some similarities to the healing responses of other living and non-living materials that are placed on a de-epithelialized skin base. Comparing this procedure to an autologous skin graft, it is expected that similar inflammatory steps would occur, but a salient difference is that the intact autologous graft contains micro vessels that would attach quickly to host tissue in a process known as inosculation, bringing nearly immediate life (within days) back to the skin that has been harvested [3]. While not obviating interface inflammation and its effects (such as hypertrophic scarring in some cases), re-vascularization of autologous tissue such as a skin graft has the net effect of reducing the period of inflammation, since stress cytokines are no longer being sent from the tissue. In the case of an intact allogeneic or xenogeneic skin graft that is applied to de-epithelialized skin, which is common in burn treatment, inosculation will occur in a similar manner, leading to an apparent healing response that is soon truncated as an immune response develops and the graft is sloughed [4]. Although this course of treatment does not lead to complete wound healing, the benefits of this application are twofold. First, the skin provides an essential moisture and infection barrier while the patient is stabilized for subsequent autografting. Second, the highly inflammatory immune response signals the underlying tissue to form a rich vascular network known as granulation tissue that serves as an optimal substrate for subsequent autografting [4].

There are many commercial products (Alloderm® from Allergan being one) that are composed of extracellular matrix proteins such as collagen and elastin (and in some cases fibroblasts and epithelium – Apligraf® from Organogenesis) that mimic the matrix component of skin grafts, whether autografts, allografts, or xenografts. By providing a milieu within which a mild immune response can operate, they often lead to the development of granulation tissue, even as they are eventually resorbed, leading to an improvement in subsequent autologous skin grafting. In addition, they have been used in nipple reconstruction to achieve some or all of the desirable features mentioned earlier, though with unpredictable and often disappointing results.

3. Effect of decellularization on healing

The entry into clinical care of the decellularized nipple allograft is therefore not without precedent, but some steps will be required to ensure that healing of such structures can proceed optimally. This begins with the preparation of the tissue itself. It has become

clear in work with other decellularized tissues that the processes of decellularization (including in some cases, terminal sterilization) vary widely and can have substantial impact on the healing properties of matrices [5]. The decellularization process must be harsh enough to remove cellular antigens, but mild enough to retain the native extracellular matrix structure, which is critical for providing the proper signaling and support structure to ingrowing cells. If decellularization is incomplete such that cellular antigens remain in immunogenic quantities, undesirable inflammation will be exacerbated, which can slow or prevent healing of the graft. But, if the extracellular matrix is damaged or crosslinked (an outcome that is particularly associated with gamma irradiation sterilization) by excessive chemical or mechanical preparation, the graft will also be less serviceable to the healing process.

Badylak points out that all scaffolds are eventually replaced by host tissue, but that depending upon modes of preparation, this can follow one of two pathways [5]. When a scaffold is decellularized to retain its native structure, contains no cellular antigens and is not from an overly aged source, it undergoes a controlled, scar-free replacement process known as “Constructive Remodeling.” Conversely, if the decellularization is incomplete, too harsh, or terminal sterilization creates unnatural crosslinking, a highly inflammatory process is instituted, leading to scar tissue formation. This is known as “Deconstructive Remodeling.” Therefore, for a decellularized nipple allograft to have the potential to be replaced *in situ* and retain its 3D structure, it will be critical that substantial attention be paid to its preparation process after recovery.

4. Healing of an externally placed acellular nipple graft

Now, assuming that such an optimal preparation has been achieved, what are the likely biological responses to a decellularized nipple allograft from the moment of attachment to a woman's breast? Initially, despite the achievement of gross hemostasis at the interface during surgery, a thin veneer of fibrin is likely to be present beneath the graft. Platelets, attached to the fibrin through their GPIIb-IIIa receptors, will have been stimulated to release an array of cytokines that will recruit neutrophils and macrophages to the scene. As the latter cells enter the area and probe the underlying surface of the graft, they will begin work in three directions. First, they will sense the need to scavenge the fibrin and platelets and will release cathepsins and other lytic peptides. Second, the macrophages will predominantly be of the M1 or pro-inflammatory types and will begin releasing immunomodulating agents to recruit additional macrophages, neutrophils and eventually, fibroblasts. Third, the macrophages will also sense the low oxygen tension at the tissue interface and emit vasculogenic cytokines such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) [5].

As capillaries begin to proliferate at the undersurface of the graft, and as debris is cleared away, these vessels will begin to probe the graft itself, seeking to attach themselves to the graft by binding to preserved extracellular matrix anchors via integrins on their cellular membranes. Blood flow will eventually contribute endothelial progenitor cells to the site, leading to vascular ingrowth along the oxygen gradient that is present. Vascular pericytes that bear mesenchymal stem cell properties will then, in conjunction with macrophages, govern the early population of the graft and augment the recruitment of fibroblasts. In addition, for a breast mound that remains sensate after reconstruction, there is the potential for axons to enter the graft in parallel with the blood vessels. If the tissue preparation has elicited a very minor inflammatory response at this stage, a macrophage conversion will occur from the pro-inflammatory M1 state to the anti-inflammatory M2 state. The latter macrophages will then begin to release substances such as

growth factors that elicit a Th2 (T helper cell) response, which “manages” the rate and spatial deposition of fibroblasts and also controls their production of extracellular matrix proteins, such as collagen, fibronectin, vitronectin and elastin, in amounts and orientations that respect the configuration of the decellularized matrix that they are slowly remodeling through replacement. This process will occur until an emerging vascularized border is present at the edge of the implant.

At this stage, epithelial cells at the border of the graft will have a toehold on the vascularized matrix and can begin to divide and migrate toward the center of the nipple as a function of the accretive vascularization of the implant. The migrating epithelial cells will likely include melanocytes when present. While fibroblast proliferation and volumetric remodeling of the graft itself continues to be governed by M2 macrophages and pericytes, the epithelium will continue to cover the nipple until this is complete. At that point, oxygen tension within the remodeled and now revascularized (and potentially re-innervated) nipple becomes normal and macrophages substantially recede, except for a population that will govern final remodeling of the nipple structure, an event that will be augmented by their mechanotransduction receptors as they are exposed to normal orthotopic breast stresses [6]. When remodeling has achieved an “equal and opposite” relationship to those breast forces, remodeling will stop and potentially, some sensation will recover. Depending upon the degree of melanocyte participation in the epithelial migration (or, depending upon conversion of epithelial cells to melanocytes), the nipple will exhibit a hue that is reflective of their participation. In some cases, nipple tattooing may be performed to augment hue after remodeling has been complete. If re-innervation does occur, it is likely that the quality of sensation will continue to mature for some time after complete remodeling.

This constitutes a putative mechanism for the engraftment of a decellularized nipple allograft that is likely to follow the principles of engraftment of other foreign, decellularized tissues, albeit in the context of a unique placement on the surface of the human body.

Ethical approval

This was not required for this work.

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Declaration of competing interest

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