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Applications of ECM Analogs in Surgery

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Abstract The loss of tissue mass in humans has been conventionally treated as an irreversible change. Treatments have emphasized replacement of the missing function by use of a transplant, an autograft, tissue synthesized in vitro or, most commonly, by use of engineering devices based on biomaterials. During the last few years solid progress has been made in the area of tissue and organ regeneration. This new approach is based on the discovery that certain simple chemical analogs of extracellular matrices synthesized by graft copolymerization of a glycosaminoglycan onto type I collagen can induce synthesis of physiologic tissue in lesions which otherwise heal spontaneously by synthesis of scar tissue. This approach offers serious potential advantages over the alternatives listed above since the graft "grows out" of host tissue. However, regeneration in the adult mammal has been successfully demonstrated so far only in skin (human, guinea pig), sciatic nerve (rat) and the knee meniscus (dog). © 1994 Wiley-Liss, Inc.

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Loss of tissue mass or of an entire organ may result from trauma or from elective surgery (eg., oncological surgery). The resulting deficit is usually a massive loss of physiologic function and is treated typically by use of an engineering device (eg., kidney dialysis machine). Other approaches to massive loss of tissue or organ mass include transplantation, autografting and in vitro synthesis of the lost tissue. Transplantation has led to certain spectacular successes, e.g., heart transplant, but it has been burdened by problems of rejection and unavailability of a donor organ. Autografting is a widely used method of treating organ loss. For instance, it is an effective procedure for the long-term treatment of full-thickness skin loss, with significant prevention of scarring and contraction, as well as being the procedure of choice in coronary artery bypass surgery. The disadvantages of autografting are the additional trauma sustained at the patient's donor site and the frequent unavailability of an intact or suitable autogenous organ for harvesting. Another procedure relies on the hypothesis that cells can, in principle, be cultured in vitro in the appropriate medium to form physiologically functioning organs that can then be implanted into the patient who donated the cells.

Replacement of organs with engineering devices fabricated from a combination of polymerics, ceramics, and metallics ("biomaterials") has enjoyed extensive use (e.g., the artificial hip prosthesis). It is, however, limited by the typically acute physicochemical and biological incompatibility of the device and the host.

A fifth route to organ repair and replacement has become available, based on evidence that diverse tissues, such as the dermis, peripheral nerve and the knee meniscus, can be regenerated if the lesions are treated with certain analogs of extracellular matrices (ECM). The analogs which have shown this unusual biological activity are copolymers formed by grafting chains from chondroitin 6-sulfate on type I collagen. Chondroitin 6-sulfate is a glycosaminoglycan (GAG) whereas collagen is a fibrous protein. Following nomenclature suggested by IUPAC [Ring et al., 1985], these polymers will be referred to as collagen-*graft*-chondroitin 6-sulfate copolymers; or generically, as collagen-*graft*-glycosaminoglycan copolymers; or briefly, as CG copolymers.

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Regeneration occurs quite differently in the various species. In amphibians, virtually complete regeneration occurs spontaneously (i.e., without assistance from templates) following amputation of a limb, provided that the amphibian has not yet undergone metamorphosis [Wallace, 1981]. At the other extreme, spontaneous regeneration of most tissues in adult mammals is not observed; instead, synthesis of nonphysiologic connective tissue, usually termed scar, occurs. In particular, several investigators have noted that regeneration of the dermis (the inner tissue layer of skin) does not occur in mammals [Billingham and Medawar, 1955; Roels, 1981; Peacock, 1984].

Several investigators, starting as early as 1943 [Schmitt, 1985; Grillo and Gross, 1962; Abbenhaus et al., 1965], have pioneered the medical use of collagen in various states of reconstitution and various geometries, in applications as diverse as skin wound dressings and surgical sutures. However, it has been shown that casually reconstituted collagens are, sooner or later, simply degraded at the lesion site without affecting the kinetics or mechanism of wound healing in any remarkable way. Only a relatively small subset of the various collagen-based ECM analogs prepared are capable of diverting the kinetics and mechanism of skin or nerve wound healing definitively away from formation of scar tissue or neuroma towards regeneration of skin and nerve, respectively, I will use the term "regeneration template," or simply template, to distinguish these highly specific extracellular matrices from other collagen preparations.

In this chapter, I summarize the evidence that supports the validity of this new approach in the experimental and clinical treatment of certain categories of organ loss and I point out certain questions that this new approach opens up.

REGENERATION OF SKIN

The skin comprises two quite distinct layers. The outer layer, the epidermis, is a multilayered cell membrane which functions physiologically as an efficient moisture seal for the organism [Yannas and Burke, 1980]. The inner layer, the dermis, is a tough, highly deformable, fiber-reinforced composite material which protects the internal organs primarily from external mechanical forces [Montagna, 1972]. When destroyed, as in a first-degree burn, the epidermis blisters, is sloughed off and regenerates spontaneously within several days provided that there is a dermal substrate over which the new epidermis can grow. By contrast to the epidermis, several investigators have stressed the absence of evidence that the dermis can regenerate [Billingham and Medawar, 1955; Roels, 1981; Peacock, 1984]. Following removal of the entire dermis from a guinea pig, for example, the rectangular wound perimeter contracts vigorously and eventually the wound edges appose each other with formation of a newly synthesized strip of scar tissue. Scar is distinctly different morphologically from intact skin.

Full-thickness skin loss in severely burned patients has been successfully treated by use of a highly porous graft copolymer of type I collagen and chondroitin 6-sulfate [Yannas et al., 1975, 1981, 1982, 1989]. Results from a 10-patient study [Burke et al., 1981] and an eleven-center 106-patient study [Heimbach et al., 1988] have been reviewed and compared with results of treatment by autografting. A histologic study [Stern et al., 1990] and an immunologic study [Michaeli and McPherson, 1990] have supplemented the results of the multicenter surgical study [Heimbach et al., 1988]. The investigators concluded that there was less hypertrophic scarring on sites grafted with the ECM analog than on sites grafted with control grafts (autografts, allografts, xenografts and a synthetic dressing). Furthermore, more patients eventually preferred the sites that were grafted with the ECM analog than those grafted with controls [Heimbach et al., 1988]. Histologic studies based on serial biopsy specimens obtained from the patients during a period of 7 days to 2 years after grafting showed that an intact dermis was achieved as well as definitive closure of a complete epidermal layer with a minimum of scarring [Stern et al., 1990]. Immunologic studies of the same patient population were based on serial serum samples obtained from patients who had grafts of the ECM analog for the determination of the humoral immune response to it. The investigators concluded that increased antibody activity to bovine skin collagen and bovine skin collagen with chondroitin 6-sulfate was not immunologically significant [Michaeli and McPherson, 1990]. The ECM analog used in these studies is supplied with an upper layer of a thin silicone membrane; the latter allows the surgeon to suture the mechanically weak ECM analog onto the woundbed and serves the purpose of maintaining physiologic moisture flow into the woundbed [Yannas and Burke, 1980].

In this surgical protocol the silicone layer is removed and disposed of on day 14 to reveal the newly synthesized neodermis on top of which is grafted the thin autoepidermal graft.

Although the therapeutic approach based on the use of an ECM analog on full-thickness wounds has received a fair share of attention by clinicians it falls short of achieving regeneration of a dermis which is physiologically complete. The new dermis synthesized in these studies lacks hair and sweat glands [Yannas et al., 1982, 1989] and these definciencies impose certain restrictions on the quality of life of treated individuals who customarily involve themselves in strenuous activity in the sun. In addition, the treatment does not lead to formation of a new epidermis and requires instead the use of an epidermal graft. In a later study the investigators seeded the ECM analog by gentle centrifugation with autologous keratinocytes and demonstrated that the seeded ECM was capable of inducing simultaneous synthesis of both a dermis and an epidermis in animals [Yannas et al., 1982, 1989]. However, this improved treatment has not been demonstrated in humans.

Insight on the structural specificity of the ECM analog can be obtained from extensive studies with guinea pigs [Yannas et al., 1975, 1981, 1982, 1989]. These studies showed that the average pore diameter and the degradation rate (measured in a standardized collagenase solution) must be controlled within narrow limits in order for the ECM analog to interfere with wound healing process in a significant way. The ECM analog modified dramatically the classical inflammatory response that eventually leads to healing with scar. The most direct aspect of this modification was the inhibition of wound contraction; the onset of the latter in full-thickness guinea pig wounds was delayed by as much as 20 days [Yannas, 1981]. The observed interference with conventional healing was decisive, leading to synthesis of physiologic tissue (regeneration) rather than to synthesis of scar tissue (repair). Healing of full-thickness wounds in the guinea pig model and in the human differs in a number of respects (e.g., contraction of the guinea pig wound is much more extensive than in the human) and the attempt to interpret the results in the human by reference to the guinea pig model must be qualified.

Evidence that the newly synthesized tissue is quite distinct from scar tissue, although not identical to intact mature skin, has been presented [Ferdman and Yannas, 1987, 1993; Yannas et al., 1988; Murphy et al., 1990]. Ultrastructural studies of intact skin, newly synthesized skin and scar show that the cellularity, constituent vasculature and neural structures allow for an easy separation of the newly formed dermis from scar. The new skin does not contain residual fragments of ECM analog. Differences between the average orientation of axes of collagen fibers in the dermis of newly formed skin and in the dermis of scar were studied by a small-angle light scattering technique [Ferdman and Yannas, 1987, 1993]. Scattering patterns obtained with tissue specimens prepared by conventional histological procedures were used to calculate a Hermans orientation function, S [Ferdman and Yannas, 1987, 1993; Yannas, 1988]:

$$S = 2\langle\cos^2\alpha\rangle - 1. \quad (1)$$

In Equation (1) α is the angle between the axis of a collagen fiber and the optical axis of the tissue section, whereas $\langle\cos^2\alpha\rangle$ implies averaging over all scattering units. The orientation index S assumes values of 0 for entirely random orientation, 1 for perfect axial orientation and intermediate values for partial orientation. The results of a guinea pig study have shown that S takes the values 0.25 ± 0.09, 0.45 ± 0.11 and 0.84 ± 0.05 for intact dermis, dermis synthesized by use of the ECM analog and scar, respectively [Yannas, 1988].

REGENERATION OF PERIPHERAL NERVE

The biological activity of CG copolymers is not limited to skin wounds. An animal study of 10-mm-long and 15-mm-long lesions in the rat sciatic nerve which were bridged with silicone tubes containing porous CG copolymers showed a clear incidence of regeneration of new, functional nerve in almost every instance [Yannas et al., 1985, 1987; Yannas, 1990]. Regenerated sciatic nerves possessed at least 60% of normal electrophysiological activity [Yannas, 1990]. Histological and ultrastructural studies have shown the presence of large numbers of myelinated axons in regenerated nerves and the absence of fragments of residual CG copolymer 6 weeks after implantation [Yannas et al., 1987]. Studies of electrophysiological activity amount to nonsacrificial assays which can be used to construct structure-property relations [Yannas, 1990].

These studies established the specific requirements of regenerating axons for certain structural features of the ECM analogs. The first feature is persistence of the ECM analog in a largely undegraded porous architecture over a period not longer than a few weeks. The second requirement refers to the architectural details of the ECM analog, including the average pore diameter and the average orientation of pore channels. The combined results suggest that elongating axons are required to make intimate contact with the solid-like surface of the ECM analog in order that regeneration be rapid and complete. However, a comparison of the structural requirements for an ECM analog which induces regeneration of the dermis and one which induces regeneration of the sciatic nerve shows certain clear differences between the two. This suggests, not unexpectedly, that ECM analogs induce regeneration in a tissue-specific manner.

REGENERATION OF THE KNEE MENISCUS

Recently, success has been reported in regeneration of knee meniscus using an ECM analog similar, though not identical, in structure to those used to induce regeneration of skin or peripheral nerve [Stone et al., 1990; Rodkey, 1992].

CONCLUSIONS

Evidence has been presented above to show that tissue regeneration using engineered analogs of extracellular matrices provides unique therapeutic alternatives to currently used therapies, including autografting, transplantation and the use of biomaterials which remain intact (or almost intact) following implantation.

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