SECTION III

BASIC SCIENCE AND PATHOLOGY

Immune Responses to Allogeneic and Xenogeneic Implants of Collagen and Collagen Derivatives

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Whereas xenogeneic collagen has provided a safe and effective biomaterial for numerous medical applications, there are few instances in which data permit the correlation of the immunologic profile of well-defined devices with their clinical sequelae. A major exception is the use of injectable bovine dermal collagen for soft-tissue contour correction. The low incidence of hypersensitivity has been studied in the context of clinical efficacy and safety with several devices. The findings indicate that such immunity usually results in the manifestation of local symptoms of dermal inflammation at sites of treatment that resolve as the implant is resorbed by the host. In contrast, more immunogenic hemostatic agents may elicit a more frequent or vigorous immune response that is not clinically visible or relevant in that application. Recent experiences with collagen-based devices for the repair and regeneration of bone have also demonstrated that the presence of immunity to their collagenous or noncollagenous components does not necessarily predict adverse clinical sequelae. Indeed, numerous specific data indicate that this immunity can exist as an epiphenomenon with no effect on osteogenesis. To get a true composite picture of biocompatibility, significant steps must be taken to characterize biomaterials properly and to ensure that immunologic, clinical, histologic, and other pertinent laboratory data are viewed in relation to one another and not in isolation.

The biocompatibility and versatility of collagen for medical applications has long been recognized and reflected in its widespread use. Several recent reviews discuss the diversity of indications treated with collagen or collagen-based medical devices.[82,87,101,107] The basis for the adoption of collagen resides in its biologic, chemical, and physical properties that permit widely diverse formulations with unique, desirable *in vivo* properties. The medical applications discussed in this article will primarily revolve around augmentation of soft tissue, treatment of acute and chronic wounds, and the repair and regeneration of bone.

Although there are at least ten genetically distinct types of collagen,[12,90] dermal collagens have been the primary source of biomaterials because of their abundance and extensive characterization. Clearly the safe use of dermal collagen as a biomaterial in medicine has long been established, most notably

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through resorbable sutures and hemostatic agents that have benefited millions of patients for decades. Though these devices are safe and effective, it has long been recognized that some subjects may develop an immunity to the collagen or other components in them. It is out of the scope of this review to represent a comprehensive presentation of the vast literature concerning collagen. This review presents an overview of devices containing dermal collagen in specific areas of medicine.

The presence of an immune response to collagen or any biomaterial is of great interest in understanding the biologic properties of that agent, but it must be viewed in the context of its clinical performance. It is not in itself predictive of clinical acceptance and must be viewed in conjunction with clinical effectiveness and sequelae. Several examples, besides sutures and hemostatic agents, will be presented to indicate that immunity to collagen can exist without impairing the effectiveness of the medical device or resulting in significant medical adverse experiences. In evaluating any biomaterial, including collagen, the immunologic data must be viewed as one component of overall biocompatibility and medical effectiveness.

SOFT-TISSUE AUGMENTATION

The collagen component of allogeneic skin grafts is not the source of sensitization or the target of rejection. Oliver *et al.*[84,85] demonstrated that cell-free collagen allografts were accepted without rejection in both domestic pig and rat models after subcutaneous implantation. Unlike skin allografts that were routinely rejected by classic mechanisms of hypersensitivity, dermal collagen allografts were infiltrated by host fibroblasts and revascularized with no evidence of lymphocyte invasion or immunologic rejection. Subsequent studies have demonstrated that even collagen-based skin-graft equivalents containing allogeneic fibroblasts can survive after transplantation without immune recognition.[64,105] Historic reports have also appeared which suggest that, after the rejection of skin allografts in human burn patients, the transplanted allogeneic collagen component of the graft can be detected in the absence of inflammation and apparently incorporated into host skin.[41] These results support the hypothesis that the major target for immunologic rejection of allogeneic skin grafts is the epidermis or the vasculature, and that the dermis, especially the dermal collagen, is accepted by the host without eliciting an immune response.

The most widely characterized collagen devices are injectable collagens (Zyderm and Zyplast Collagens, Collagen Corporation, Palo Alto, California), which are used for the correction of soft-tissue contour irregularities caused by aging, trauma, disease, and congenital deformities. There have been numerous descriptions of preclinical and clinical results with these collagen devices, and the following discussions of biocompatibility,[14,51,56,74,93,111] efficacy,[22,34,55,110] and immunology[23,24,26–29,33,72,106] are a representative overview. Early large-scale clinical studies with injectable collagens verified that approximately 3% of the population develops hypersensitivity to the initial skin challenge with injectable collagen,[14,22,51] and since most of these reactions occur within the first 72 hours,[22,28] it indicates a presensitization to bovine collagen, presumably due to dietary exposure. In addition, approximately 1% of subjects subsequently treated with these injectable bovine collagens will develop localized hypersensitivity responses.[14,22,29,51] Antibodies to bovine collagen correlated with these local, inflammatory reactions,[23,24,29,33,72,106] and the histologic picture was typical of an immune reaction in the skin with a lymphohistiocytic infiltrate and scattered plasma cells.[110] The clinical manifestations of these hypersensitivity reactions have been well described and are predominantly erythematous, indurated regions surrounding some or all sites of bovine collagen injection.[29,110] Characterization of the immune response in these subjects has been actively pursued.[23,24,26–29,33,72,106] Anti-

bodies found in their sera are specific for bovine collagen and do not cross-react with human collagen[24,29,33] (Fig. 1). These antibodies recognize antigenic determinants shared by all of the bovine interstitial collagens, apparently representing species-specific epitopes in bovine collagen,[33] and they can be found *in vivo* bound to bovine collagen at these hypersensitivity sites (Fig. 2). Immunohistology demonstrates host antibody in infiltrating plasma cells and bound to bovine dermal collagen implants but not to surrounding host dermis (Figs. 2D and 2E). The precise role of cell-mediated immunity in these reactions and its balance with the humoral response remains to be clearly elucidated. Thus, these data have characterized the incidence and character of hypersensitivity reactions in subjects treated with these collagen implants and these localized inflammatory reactions, associated with an immune response specific for bovine collagen, resolve as the implant is resorbed.

Several recent publications discuss the immune response and biocompatibility of other collagen-based devices. In the initial publication on one such device, Fibrel implant (Serono Laboratories, Randolph, Massachusetts) which contains denatured collagen,[76] inflammatory reactions that could have represented immunologic responses were presented but were not evaluated as such. Although very limited serologic studies were performed, the data were not presented in detail or related to adverse experiences. In a later publication on long-term follow-up evaluations in these subjects, the immunologic studies were not included, and the conclusion that no hypersensitivity reactions had occurred was not supported by immunologic verification.[77] Accurate assessments of the immunologic properties of this biomaterial and its propensity to elicit hypersensitivity reactions will have to await adequate studies in the future.

Using injectable bovine collagen (Atelocollagen, Koken Company, Tokyo, Japan) for the correction of contour irregularities in soft tissue, Charriere *et al.*[16] conducted a large study which related clinical hypersensitivity to antibodies to collagen. That study found that the incidence of hypersensitivity and the characteristics of these responses were similar to those previously described for Zyderm

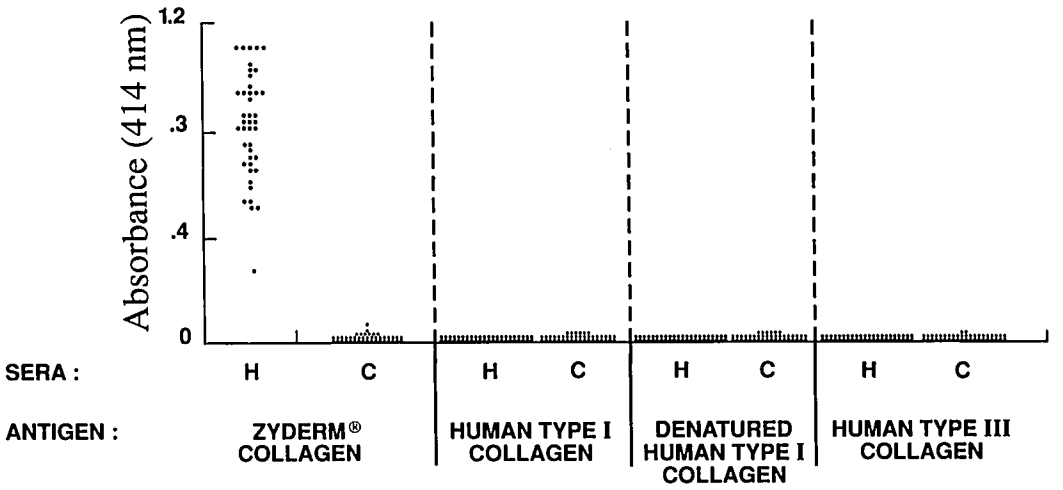
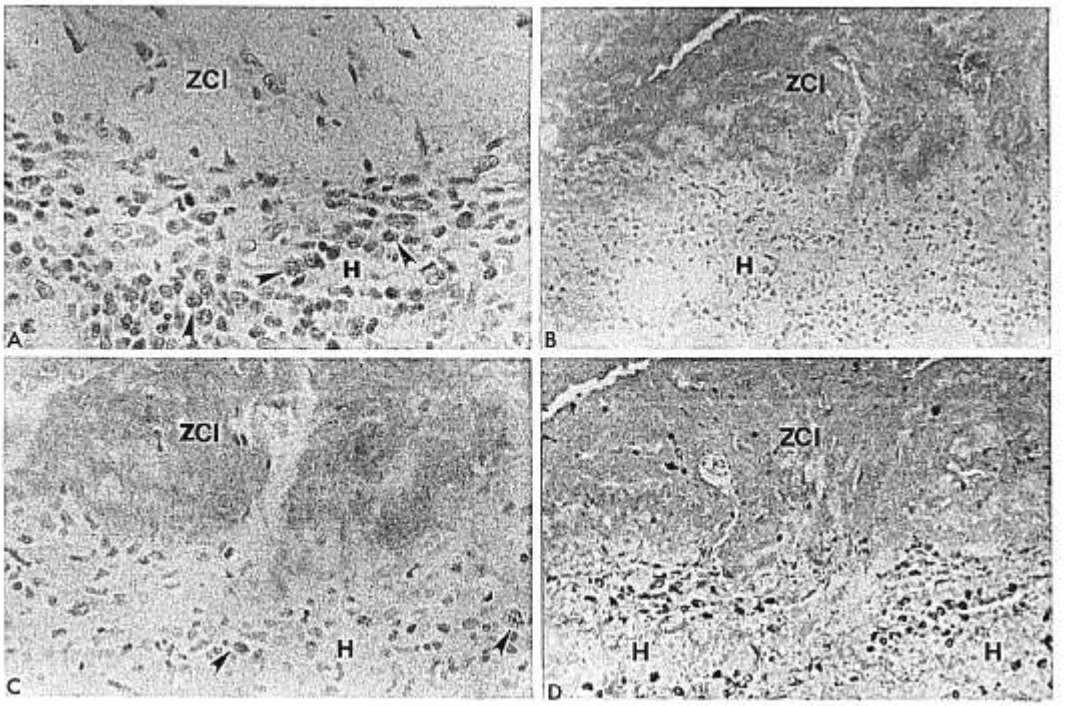


FIG. 1. Sera from 42 subjects with hypersensitivity (H) to Zyderm Collagen and from 42 healthy control subjects (C) examined by ELISA for antibodies against Zyderm Collagen, human Type I collagen, denatured human Type I collagen, and human Type III collagen. Results are expressed as absorbance values (414 nm) at 1:4 sera dilutions.



FIGS. 2A–2E. Using immunohistologic techniques, sections from a biopsy specimen of a subject experiencing a hypersensitivity reaction to Zyderm Collagen Implant (ZCI) were examined for the presence of *in situ* antibodies. (A) The absence of immunostaining of ZCI or surrounding host dermal collagen (H) is demonstrated in this section, which was treated with normal rabbit serum followed by a biotinylated goat antirabbit antibody serum (BGARS) and strepavidin–peroxidase (SP). The final substrate was diaminobenzidene (DAB). (B) The staining of the bovine collagen implant (ZCI) is demonstrated in this section, which was treated with rabbit anti-Type I bovine collagen serum followed by BGARS and SP. The final substrate was also DAB. (C) When the section from (B) is viewed at higher magnification, the presence of immunoglobulin-producing plasma cells (arrows) in the local inflammatory infiltrate becomes clearer. (D) *In situ* binding of the subject's antibodies to the ZCI but not to the surrounding host collagen (H) is seen by treating sections with rabbit antihuman immunoglobulin antibodies. This is followed with BGARS and SP, as before, and finally a DAB substrate. (E) With the section seen in (D) at higher magnification, it is easier to distinguish the presence of antibodies within locally infiltrating plasma cells (arrows). (Stain, 1% methyl green; original magnification, ×400 in A, C, and E, ×200 in B and D.)

Collagen. They found that localized inflammatory reactions occurred most frequently after initial exposures and that the reactions resolved with time as the implant was resorbed. The authors, however, discontinued treatment in five subjects who demonstrated antibodies to bovine Type II collagen because they believed this indicated an ongoing rheumatologic disease. Although they did not associate this finding with the biomaterial used, it represents an unfounded leap from *in vitro* antibody results to clinical conclusions. It is clear that in many cases antibodies that react with bovine Type II collagen do not cross-react with human Type II collagen.[33] Clinical studies have also shown that antibodies to several types of human collagen do not display disease specificity and appear to represent a secondary response to tissue injury.[113] In addition, antibodies to human Type II collagen have not been proven to correlate with disease activity in patients with rheumatoid ar-

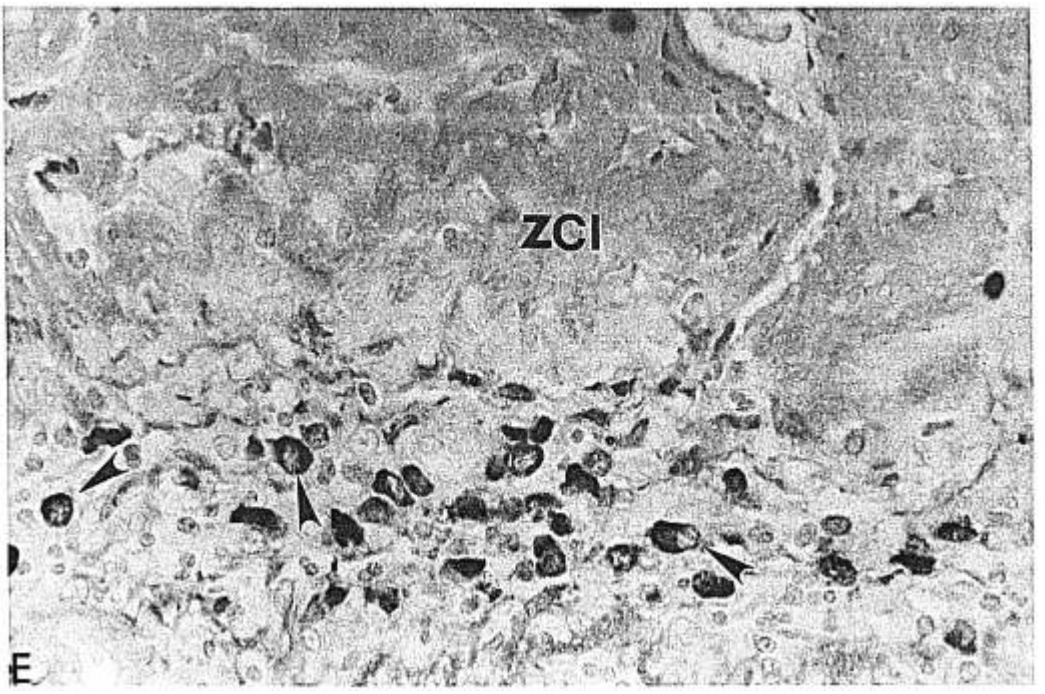


FIG. 2 (*Continued*).

thritis,[80] and studies in mice have demonstrated that, although some strains of mice can develop high titers of antibodies to Type II collagen, they do not develop symptoms of disease.[128] The development of an arthritic disease in certain other strains of mice is highly dependent on immunity to unique arthritogenic determinants in Type II collagen that are not found in Types I or III collagen.[116,117] Thus, while Charriere *et al.*[16] accurately related clinical performance of the implant with immunologic status of the recipients, it is erroneous to assume that antibodies to bovine Type II collagen are inherently indicative of existing or incipient rheumatologic disease.

In a recent publication, a comparative study utilized human skin and amnion collagens in a rat model.[66] The skin-derived biomaterial was both more inflammatory and more immunogenic than the collagen preparation obtained from amnion. The conclusion that the basis for these differences resides in the ratios of Types I and III collagen is premature. The *in vitro* antibody assessments were performed only with these skin and amnion preparations. The data do not rule out the likely presence of immunogenic contaminants such as pepsin or human albumin, which may be contributing to the inflammation and immunogenicity.[129] Additional assays with a panel of purified antigens, including the relevant types of collagen, are essential. It is also essential to understand that the irradiated collagen preparations used in this study represent modified collagens, not intact native materials. Since it is well known that irradiation results in significant modifications (both denaturation and cross-linking) in the collagen molecule and affects biologic performance, equating these materials to nonirradiated, native collagen devices is misleading. Comparisons of biomaterials, including collagen devices, must account for process differences that can have an impact on biocompatibility and immunogenicity.

WOUND REPAIR

It has been reported that collagen is less antigenic than most proteins and that the major antigenic epitopes reside in the nonhelical telopeptide portion of the collagen molecule.[25] Takeda *et al.*[114] compared the relative immunogenic potentials of bovine collagen wound dressings prepared from tropocollagen and collagen obtained by selectively removing the telopeptide atelocollagen. Cell-mediated immunity and humoral immunity were evaluated in rabbits, whereas immunoglobulin E (IgE) antibody production was assessed using sera from hyperimmunized mice in a passive cutaneous anaphylaxis (PCA) model.[114] In brief, wound dressings prepared from atelocollagen did not induce either an Arthus reaction or a delayed type hypersensitivity (DTH) response after intradermal skin testing. In contrast, wound dressings composed of tropocollagen promoted both an Arthus reaction and a DTH response after skin challenge in rabbits. The heterologous PCA assays were negative, indicating that neither tropocollagen nor atelocollagen elicited IgE antibodies. These observations suggest that dressings prepared from atelocollagen are less antigenic than dressings prepared from tropocollagen.[114]

The loss of skin in the burn patient poses a significant support problem until the wounds have been closed. Artificial skin and collagen membranes have been used as a short-term skin substitute in the form of wound coverings.[115,121] For example, collagen-rich pigskin and amniotic membranes have been used as temporary coverage in the management of burn wounds.[18,91,94,114] The collagen component of artificial skin appears to have low immunogenic potential, since the nonhelical telopeptide regions of the collagen molecule contribute primarily to its antigenicity.[2,3,31,95,118]

Collagen membranes have been used successfully to cover split-thickness donor graft sites in burn patients[91] and are comparable to synthetic dressings. Thin (0.06 mm) bovine collagen membranes were advantageous in diminishing blood loss because of their hemostatic properties. Donor sites treated with collagen membranes exhibited improved healing and a modest decrease in pain compared with synthetic dressings.[91] A slight inflammatory response has been reported in association with collagen dressings used in wound sites.[13,99,102] However, these studies do not adequately describe the nature of the inflammation or characterize any immune responses to the collagen dressings.

The hemostatic properties of collagen are useful in controlling diffuse bleeding over large surfaces. The fibrillar collagen network entraps platelets, promotes their aggregation, and subsequently triggers degranulation to form a fibrin plug.[20,43,44,71] Collagen has been used as a hemostatic agent in split-thickness donor sites,[70,91,127] as well as in liver, spleen, and maxillofacial surgery when individual blood vessels cannot be easily clamped.[4,17,92,96] Although these collagen devices appear to have low immunogenic potential with minimal adverse effects,[11] moderate inflammatory responses have been observed in adjacent tissues after application of the hemostatic agents. This inflammatory response has been characterized as a granulomatous, foreign-body reaction.[73] The immune responses to several of these biomaterials have been characterized and compared with other collagen-derived medical devices in multiple animal models.[26,29] Noncollagenous contaminants, most notably bovine serum albumin, were the most immunogenic components of one hemostatic agent, a microcrystalline collagen.

The use of a human-collagen fleece as a hemostatic agent has been recently reported.[58] In 63 patients treated with human collagen, no adverse effects were reported. Human collagen hemostatic agents may prove superior to those derived from bovine collagen, provided adverse responses are minimal or nonexistent.

Collagen materials have been used in several ophthalmic applications. Resorbable collagen sutures have been widely used in corneal surgery, with few adverse experiences.[108] Purified collagen films (0.0013 mm thick) have been used in keratoplasty to replace portions of the cornea excised after full-thickness chemical burns in cats. The collagen films formed a watertight seal and became translucent. Examination of the treated corneas at five months postoperatively demonstrated normal features with no evidence of inflammation, and serum antibodies against the collagen implants were not generated.[5] Aqueous collagen solutions have been used as a vitreous replacement in animal studies. The collagen implant appears to be degraded in a relatively short period of time and replaced by the animal's own vitreous.[32] In that study, mild inflammation was observed, but the inflammation did not result in damage to the eye. That report did not characterize the inflammatory reaction nor was there a characterization of the immune response to the collagen. Stenzel *et al.*[112] evaluated the therapeutic utility of calf-skin collagen solutions as a vitreous replacement in rabbits and nonhuman primates. In those studies, a mild inflammatory response was observed immediately after injection of the collagen solution into the vitreous but was reported to be transient, resolving within five to ten days after the treatment. Histologic examination revealed no evidence of inflammation within the vitreous and apparent resorption of the collagen within two months. Again immunologic assessments were not performed.

The biocompatibility of tape composed of purified bovine collagen has been histologically evaluated in sclera, cornea, suprachoroidal space, and the anterior chamber of the eye in cats and rabbits.[65] In general, bovine collagen was biocompatible and elicited only a mild inflammatory response. Within the sclera, a slight neutrophil infiltrate was observed within two days of implantation. The inflammation became predominantly mononuclear by two weeks; at one and two months, implant-associated giant cells and scattered plasma cells were observed. The implant appeared to have been resorbed by four months. Similar, although reduced, inflammatory responses were observed in the cornea, suprachoroidal space, and the anterior chamber of the eye. Although a mild inflammatory response to the collagen implant was observed, it was concluded from gross observation that the collagen implant did not produce a clinically significant reaction. Immunologic evaluations were not performed in those studies.

Collagen films have been successfully used in neurosurgery as dural replacements and for hemostasis.[45,49,61] Although no immunologic assessments were performed in those studies, a mild, transient foreign-body reaction was reported to the collagen film.[49] These implants were resorbed and replaced by host connective tissue at six to eight months after implantation. Collagen films have also been used to wrap severed peripheral nerves in chimpanzees. The collagen provided a scaffolding for the formation of host connective tissue around the repaired nerve, and no inflammation was reported.[57]

Collagen membranes are useful in the repair of tympanic membranes. In a dog model, collagen membranes did not promote infection or an inflammatory response.[1] Although the data were not presented, the authors concluded that there were negligible antibody titers against the bovine collagen implant.[1] The collagen implants were eventually replaced by host connective tissue and regenerated epithelial and mucosal layers.[1,50]

REPAIR AND REGENERATION OF BONE

Autogeneic bone grafting is standard practice for the treatment of bony defects resulting from injury when there is significant risk of nonunion during the healing process.

Whereas autogenous bone is the current preferred grafting material, patients may not have sufficient donor site bone or be in suitable health to be their own donors. In addition, the second surgery site always carries the risk of associated morbidity and mortality. For these reasons, various bone-graft substitutes have been explored, and the earliest substitutes were xenogeneic or allogeneic bone transplants. Since xenografts and allografts are composed in large part of collagen and mineral, their effectiveness as graft materials relate to the utility of purified collagen as a graft material. In addition, these grafts contributed considerably to the understanding of conductive and inductive bone growth.

As early as 1889, Senn[104] investigated the use of xenogeneic bovine tibias, which were decalcified, cut into small pieces, and disinfected with alcohol. These devitalized xenografts were utilized for a variety of indications, primarily for the treatment of osteomyelitis. More recently, Kiel (Unilab, Hillside, New Jersey) bone splinters, derived from young bovine or porcine bone, have been used as a graft material.[68,69] This bone was cut, deproteinated with peroxide, delipidated, and vapor sterilized. According to several clinical studies, Kiel bone was accepted and effective in new bone formation.[7,67] In 1970, however, Schweiberer *et al.*[103] reported that Kiel bone served no purpose and was actually detrimental to new bone formation. More recently, Salama[97] and Salama and Weissman[98] found that mixtures of Kiel bone and autogeneic bone marrow could be used clinically. Boplant (E. R. Squibb and Sons, Princeton, New Jersey), also made from bovine bone,[6] was detergent extracted, delipidated, chemically sterilized, freeze-dried, and packaged. Several early studies suggested that Boplant was a very efficacious material,[6,52] but its utility was called into question. Kramer *et al.*[60] found that implants and onlays of Boplant led to a massive influx of neutrophils, followed by giant cells that caused implant resorption. Unsatisfactory results were also reported in a dog segmental defect model.[47] The variability in the results apparently stemmed from the difficulties in manufacturing such a complex material as xenogeneic bone and the presence of noncollagenous immunogenic proteins contaminating the collagen–mineral preparations.

Examination of allogeneic bone transplants demonstrated immune responses mounted against the cellular antigens in the graft.[15,37] However, the allogeneic transplants appeared to behave much as autogenous bone grafts for the first week after surgery. Thereafter, the immunologic response to the allograft caused the breakdown of vascular anastomoses between the transplant and the host vascular bed. Acellular allogeneic bone matrix, on the other hand, provided a comparatively weak immune response. Freeze-drying of allogeneic bone transplants gave improved results,[15,46] although Langer *et al.*[62] found no difference between fresh and frozen allogeneic bone transplants. After comparing the immunogenicity of fresh, frozen, and freeze-dried allogeneic transplants either with or without bone marrow,[35] the only regimen that reduced immunogenicity was freeze-drying. However, freeze-drying also had a negative effect on the osteogenic potency of allogeneic transplants. Despite the obvious immunologic complications of allogeneic bone transplants, they have been used in clinical practice by a number of surgeons with varying degrees of success.[59,109,120] Clearly, the use of allogeneic bone as a graft material has shown effectiveness when a balance is found between reduced immunogenicity and maintenance of inductive entities within the matrix. Urist[123] described the use of autolyzed, antigen-extracted, allogeneic freeze-dried bone in which such a balance is struck. However, because of concerns about sterility, the possibility of viral contamination, and the immunogenicity of allogeneic bone, still other alternatives to the autogenous bone graft have been examined. Of interest to this review is the use of collagen-containing bone graft substitutes.

Type I collagen is the major structural protein in bone, comprising approximately 90% of its organic matrix.[124] Thus, it is not sur-

prising that collagen has been used by a number of investigators for bridging bony defects[19,30,126] or as a coating for ceramic[10] or metallic implants.[119] In those studies, collagen provided a scaffold for the ingrowth of bone-forming cells, resulting in new bone formation: it served as a good conductive matrix. In addition, collagen hemostatic agents have long been used in a number of bony sites with little evidence of adverse responses.[8,20,21,48,63,86] Fabinger *et al.*[38] emphasized that the outcome of the use of collagen sponges as a graft material relies heavily on the purity of the materials used, since noncollagenous contaminants significantly enhance implant immunogenicity and inflammatory potential.

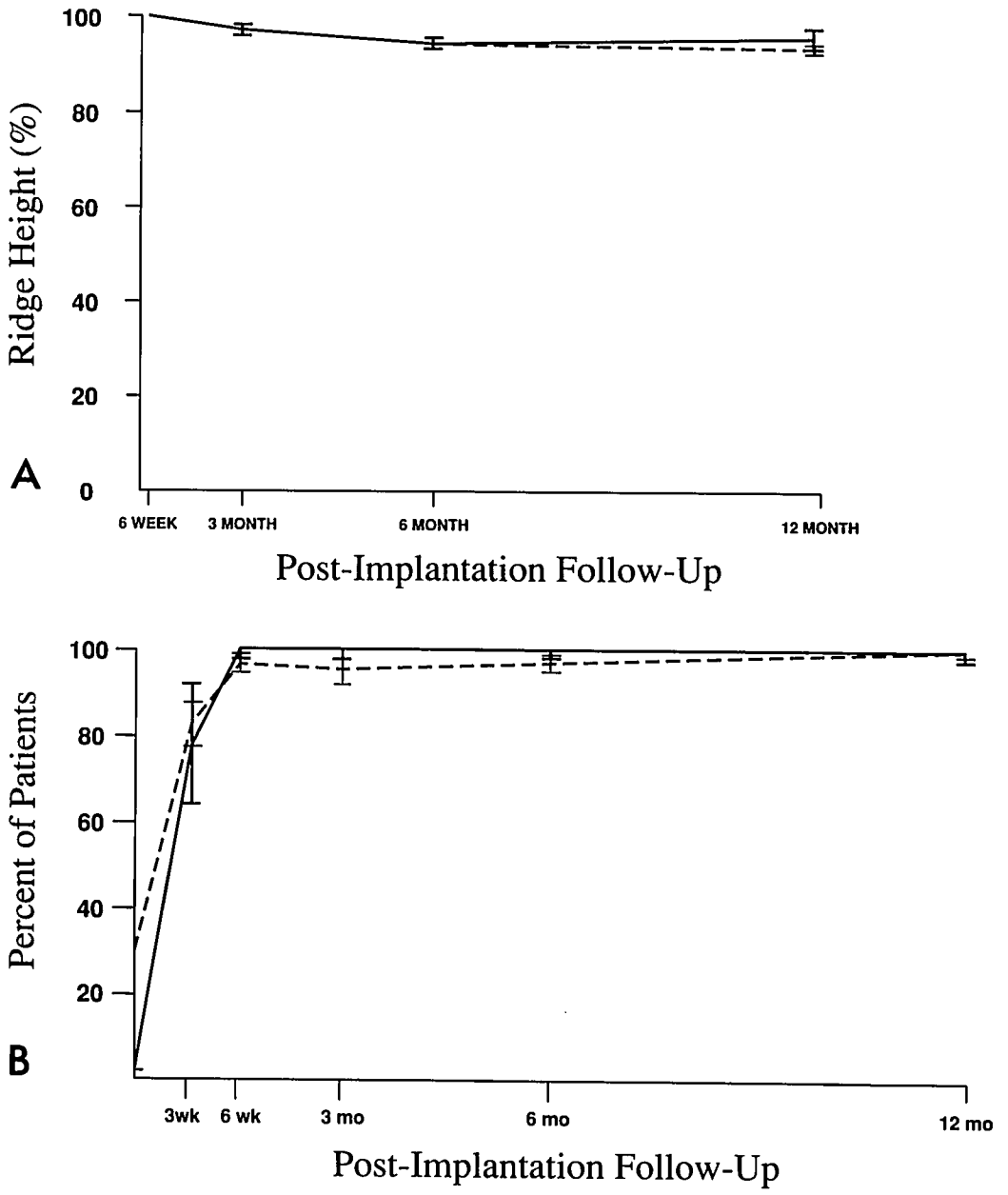
Colipat (Oscobal, Switzerland) implants were first conceived by Mittelmeier *et al.*[78] and consisted of two commercially available reagents, a collagen hemostatic agent and hydroxylapatite. The collagen was derived from porcine skin; it was digested with trypsin, dissolved, freeze-dried, and radiation sterilized. Hydroxylapatite was approximately 90% pure with the remainder being tricalcium phosphate. The collagen was cross-linked using formaldehyde and mixed in a 5:1 ratio (collagen:hydroxylapatite). Both animal and clinical studies have been performed with this material.[53,54,78] Histologically, the material appeared to be noninflammatory and biocompatible with vigorous new bone formation. However, no serologic examination of anticollagen antibodies was made in those studies, and an assessment of its immunogenicity has not been reported.

The biocompatibility of collagen in allowing adjacent new bone growth is well illustrated in a study performed with Periogen (Collagen Corporation) collagen membrane in a periodontal dog model.[89] In that study, the authors note that "there is a high degree of osteogenesis adjacent to the fully, intact cross-linked membrane" three weeks after surgery. The collagen membrane also prevented epithelial cell downgrowth, thus allowing the regeneration of lost tissue around the tooth. This material has been tested clinically in 59 patients with no adverse responses.

Serologic assessments of anticollagen antibodies have been made in studies of two collagen-based bone substitutes and are described below. The incidence and titers of anticollagen antibodies were low in these studies. In addition, the presence of antibodies against collagen did not affect clinical outcome or result in adverse clinical sequelae.

Alveoform (Collagen Corporation) biograft is a bone-grafting material for the augmentation and reconstruction of atrophic mandibles and maxillae of edentulous patients. It is composed of a mixture of purified fibrillar collagen derived from bovine dermis and hydroxylapatite particles. Upon wetting with blood *in situ*, the dried material is malleable and will conform to contours of the existing jaw bone. In clinical trials with this material,[75] there were no significant adverse responses to the biomaterial, and 100% of the patients showed good to excellent graft solidification and stabilization three months after surgery. Twenty-four months postoperatively, 97% of the patients had good to excellent alveolar ridge firmness, a key measurement of implant success. The sera of these 77 patients were evaluated for antibodies to bovine collagen using an enzyme-linked immunosorbent assay (ELISA). Examination of presurgery sera showed that five patients had evidence of preexisting antibodies to bovine collagen and five additional patients exhibited antibodies after surgery. The presence of circulating antibodies to bovine collagen was not associated with any effect on ridge augmentation (Fig. 3A) or ridge firmness (Fig. 3B). In addition, the nature and incidence of adverse clinical experiences were the same among subjects with or without antibodies to the implant for over two years of postsurgery clinical follow-up evaluations. This suggests that the presence of either preexisting or induced immunologic responses to bovine collagen are of little importance to the clinical efficacy of this implant material.

Studies have also been initiated with another biomaterial, Collagraft (Collagen Cor-



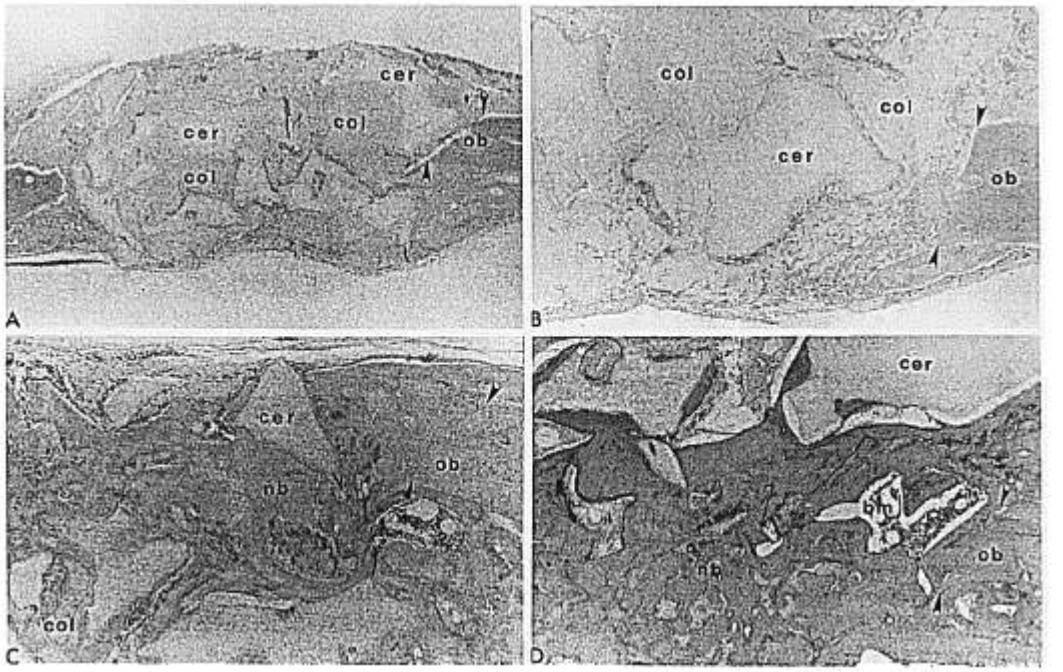
FIGS. 3A AND 3B. (A) Alveolar ridge height after denture loading and (B) alveolar ridge firmness for the Alveoform biograft. There are no significant differences between alveolar ridge height and firmness observed in subjects with (solid line) or without (dashed line) antibodies to bovine collagen following treatment with Alveoform biograft. Data on ten subjects with circulating antibodies to bovine collagen were available for (A) and nine subjects for (B), whereas 57 subjects without antibodies to bovine collagen were followed for ridge height (A) and 64 subjects for ridge firmness (B). Data are expressed as the mean ridge height (%) per group (±SD) following denture loading at six weeks (100%); the results in (B) represent the mean percentage of patients per group (±SD) who had good or excellent ridge firmness at the indicated follow-up examination.

poration, and Zimmer, Warsaw, Indiana) bone-graft substitute, which is composed of purified fibrillar collagen (PFC), a pepsin-extracted bovine dermal collagen, and hydroxylapatite/tricalcium phosphate (HA/TCP). The collagen and ceramic are mixed by the surgeon with autogenous bone marrow; the bone marrow provides a source of osteogenic progenitor cells.[40] The HA/TCP has been investigated as a bone graft substitute in its own right[79,81,88] and in conjunction with bone marrow.[83] The HA/TCP is biocompatible and has no deleterious effect on the healing of defects when mixed with cancellous bone.[79] The mixture of collagen and HA/TCP reportedly augmented serum levels of anticollagen antibodies in one study.[39] However, the ELISA data were presented only at a 1:20 dilution of sera and thus actual titers were not stated. In addition, the collagen used in that study[39] was apparently not pepsinized to remove telopeptide regions of the collagen, so it is unclear what epitopes were recognized by the elicited antibodies or if indeed noncollagenous contaminants were present and acting as immunogens.

An experimental model to assess the efficacy and safety of Collagraft was initiated in dogs.[42] A unilateral 2-cm diaphyseal defect was created in 18 mongrel dogs, and a 2-mm intramedullary Steinmann's pin was used to align the ulnar segments. In addition, a 9.5-mm cylindric defect was created in the ipsilateral proximal humerus by a trephining procedure. The contralateral sites were used as controls. Three experimental groups were defined: PFC with autogeneic bone marrow, Collagraft and bone marrow, and Collagraft alone. All dogs were evaluated roentgenographically at four-week intervals and by histologic and biomechanical means at 24 weeks after surgery. Sera were also taken from the dogs at monthly intervals. By 24 weeks, roentgenograms indicated that union had formed in all dogs of each group, except two within Group I (PFC and bone marrow). Histologically, all sections showed the entire graft site to be filled with sheets of well-organized, normal-appearing lamellar bone. This lamellar bone appeared to be remodeling into cortical bone, particularly at the periphery. The two nonunions in Group I were fibrous and were attributed to excessive motion in the defect site during healing since the Steinmann's pin does not afford much fixation. The micrographs showed intimate contact between ceramic and newly formed bone, indicating good biocompatibility. Other than normal marrow elements, no inflammatory cells were seen. Four dogs developed low but significant titers of 160 to 320 to either native or denatured bovine collagen, but these subsided by 24 weeks. Since all animals receiving Collagraft formed unions, the presence of antibodies to collagen was not significant to the clinical outcome for this bone-graft substitute and had no significant adverse effects on osteogenesis.

Collagraft bone-graft substitute was also examined in a rat parietal defect model. Figure 4 shows the histologic assessment of this material at seven, 30, and 90 days after placement. At the earliest time, no evidence of an inflammatory reaction was seen against either the collagen (PFC) or the HA/TCP. The apposition of new bone and ceramic is clearly evident at 30 days, indicating good biocompatibility. Residual collagen (PFC) could be seen at 30 days, but by 90 days the PFC had been completely replaced by new bone.

In clinical trials with Collagraft, sera from 117 individuals who received autogenous bone grafts (controls) and 134 patients treated with Collagraft were examined with ELISA for antibodies to collagen. None of the sera from control patients displayed antibodies to bovine collagen, and ten patients receiving Collagraft (7.5%) had antibodies to bovine dermal collagen (Table 1). One of these subjects demonstrated pretreatment and follow-up antibodies to denatured bovine dermal collagen only and is not included in Table 1. The maximal antibody titers ranged from 160 to 640. Of the nine patients who were evaluated at 12 months, the titers of eight subjects had dropped to borderline



FIGS. 4A–4D. Histologic evaluations were performed on Collagraft implants in a rat parietal defect at (A) and (B) 7, (C) 30, and (D) 90 days postimplantation. The cut ends of the bone are marked with arrows. The presence of various elements is marked as follows: Cer, HA/CTP particles; col, collagen; ob, old bone; nb, new bone; and bm, bone marrow. At seven days (A) and (B), the Collagraft implant is largely intact with both collagen and HA/CTP particles evident between the cut ends of the parietal bone. No inflammatory reaction is evident (B) around either the HA/CTP particles or collagen. At 30 days (C), the presence of newly formed bone is found between the cut edges of the parietal bone and the HA/CTP particles. Some residual collagen is evident at this time. By 90 days (D), whereas the cut edges of the parietal bone are still discernable, newly formed bone is found throughout the implant site. HA/CTP particles are evident but collagen is not observed. The new bone contains nacently formed bone marrow. (Stain, hematoxylin and eosin; original magnifications, ×200 in A and ×400 in B through D.)

levels by 12 months after treatment. None of these ten patients demonstrated antibodies against human Types I or III collagen (data not shown). Three of the ten had postoperative complications. The first involved wound dehiscence that resolved without complication; the second resulted in a malunion at 90 days with limited elbow motion, and the third noted infection at the incision site, which resolved with antibiotic treatment. These postoperative complications were unrelated to antibodies against bovine collagen and occurred at the same frequency among patients treated with autogenous bone. Thus, the clinical results indicated that most individuals do not develop an immunologic reaction to the collagen implant (92.5%), and even in those individuals who do develop antibovine collagen antibodies, no effect on implant efficacy or safety was observed. By a number of clinical parameters, Collagraft appears to have performed equivalently with autogeneic bone grafting in terms of efficacy and safety in the treatment of fresh fracture defects that require bone grafts.

TABLE 1. Titers of Antibodies to Bovine Collagen in Sera of Patients Treated With Collagraft: ELISA Results

		Serum Collection*			
Patient	*Antigen*	*Pretreatment*	*1.5*	*3*	*12*
1	PFC	0	320	160	40
	BI	0	160	40	0
	BIII	0	320	160	0
	DPFC	0	0	0	0
2	PFC	0	160	80	0
	BI	0	0	0	40
	BIII	0	40	0	40
	DPFC	0	320	0	40
3	PFC	0	0	0	0
	BI	0	0	0	0
	BIII	0	0	0	0
	DPFC	0	0	320	0
4	PFC	0	160	640	160
	BI	0	80	320	0
	BIII	0	160	640	0
	DPFC	0	0	0	0
5	PFC	0	640	NS	40
	BI	0	320	NS	40
	BIII	0	640	NS	80
	DPFC	0	0	NS	0
6	PFC	0	0	80	NS
	BI	0	0	80	NS
	BIII	0	0	320	NS
	DPFC	0	0	0	NS
7	PFC	0	320	320	0
	BI	0	40	160	0
	BIII	0	0	80	0
	DPFC	0	0	0	0
8	PFC	0	80	80	0
	BI	0	80	160	0
	BIII	0	80	80	0
	DPFC	0	0	0	0
9	PFC	0	80	NS	0
	BI	0	160	NS	0
	BIII	0	160	NS	0
	DPFC	0	0	NS	0

PFC, purified fibrillar collagen; BI, bovine Type I collagen; BIII, bovine Type III collagen; DPFC, denatured purified fibrillar collagen; NS, no sample.

* Sera obtained at the indicated number of months after treatment.

Early studies on xenografts and allografts clearly indicated that immunologic reactivity could limit the effectiveness of the graft.[36] Despite the limitation of these grafts, they have been widely used. The immunogenicity of these preparations is due to cellular or plasma protein contamination or both and not to the purified collagen–mineral bone matrix. The presence of inductive bone growth factors in the grafts undoubtedly has contributed to their partial success.[122] With the purification of several factors that are reported to induce osteogenesis actively,[9,100,125] defined recombinant proteins can be mixed with highly purified conductive carriers such as collagen and hydroxylapatite. This next generation of bone-graft substitutes is likely to surpass the autograft as the ideal bone-graft material. For the time being, conductive matrices, in which the regenerative potential of autologous bone marrow is combined with biocompatible ceramic and collagen, are at least equivalent to autologous bone for the treatment of bony defects, such as fresh fractures, and they can eliminate the morbidity of donor-site surgery.

CONCLUSIONS

Collagen and collagen derivatives are of unparalleled value in the development of biocompatible medical devices for a wide spectrum of applications by most medical specialties. Historically, allografts of soft and hard tissue composed predominantly of collagen have provided solutions to the needs of surgeons for the repair of defects in most major organ systems. By taking advantage of the availability of purified xenogeneic collagen and its high degree of biocompatibility, it has become possible to provide novel and innovative medical devices that can augment both soft tissue and bone, in some instances avoiding the shortage of allograft materials or sparing the patient from the need for surgery to obtain autografts.

Whereas an immune response to xenogeneic collagen has been demonstrated in both animal and human models, the data clearly demonstrate that immunity per se is not associated with significant adverse sequelae *in*

vivo. Indeed in most cases the presence of antibodies to xenogeneic collagen is an epiphenomenon and not a source of implant rejection. In the future there must be adequate documentation of the composition and purity of biomaterials utilized in experimental studies and subsequently of the antigens employed *in vitro.* In the evaluation of medical devices and biomaterials, including those containing collagen and collagen derivatives, the current literature makes it clear that it is essential to evaluate the immune response in the context of the histologic and clinical results of implants in representative animal and clinical models.

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