Antigenicity and Immunogenicity of Collagen

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Abstract: Pertinent issues of collagen antigenicity and immunogenicity are concisely reviewed as they relate to the design and application of biomedical devices. A brief discussion of the fundamental concepts of collagen immunochemistry is presented, with a subsequent review of documented clinical responses to devices containing reconstituted soluble or solubilized collagen. The significance of atelocollagen, concerns regarding collagen-induced autoimmunity, and other relevant topics are also addressed in the context of current understanding of the human immune response to collagen. © 2004 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 71B: 343–354, 2004

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INTRODUCTION

Despite its widespread acceptance as a safe and multifunctional material,[1,2] the status of collagen as an animal-derived biomaterial has always raised—and will likely always raise—concerns regarding its potential to evoke immune responses. Although the clinical incidence of adverse reactions to acellular collagen implants is exceedingly rare, they do indeed occur,[3–8] and thus an understanding of their mechanisms is essential for the design and application of new biomedical devices.

A large proportion of the literature addressing the immunochemistry of collagen was assembled in the 1960s and 1970s, and observations from many of these investigations have formed the basis for countless immunological and biochemical studies ever since. In the field of applied biomaterials, however, use of certain selective interpretations of these studies (in particular, to infer generalizations regarding biocompatibility) has become increasingly frequent, suggesting that a current review of the pertinent issues would be beneficial.

The present review attempts to summarize the key aspects of collagen antigenicity and immunogenicity as they relate to the design and clinical application of biomedical devices. It should be noted that the treatment presented here focuses on antigenicity and immunogenicity as they relate to devices comprised of reconstituted soluble or solubilized collagens; discussion of decellularized grafts containing insoluble collagen—including demineralized bone matrix[9,10] and xenogenic heart valves[11,12]—is documented elsewhere, and is included only where pertinent. The reader is referred to Yannas[13] for a comprehensive review on the structure and material properties of collagen, and to the pertinent sections of Friess[14] for a concise and current overview of collagen extraction methods. A thorough summary of the seminal immunological studies on collagen can be found in Furthmayr and Timpl.[15]

MECHANISMS OF ANTIGENIC AND IMMUNOGENIC RESPONSES TO COLLAGEN

Until 1954, collagen was largely considered to be nonimmunogenic,[16] and despite subsequent evidence demonstrating its ability to interact with antibodies, it is still considered to be a weak antigen.[2,15] Although the interpretation of immunochemical reactions to collagen-containing implants is often complicated by the presence of noncollagenous proteins,[17] cells and cell remnants,[18,19] and artefacts from crosslinking treatments,[20–22] the wealth of literature devoted to the immunological behavior of collagen itself provides an extensive basis on which such interpretations can be based.

In addressing the immunochemical properties of any protein, it is pertinent to distinguish between the potentially ambiguous terms "antigenicity" and "immunogenicity." In

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the absence of standardized methods for purification and characterization of reconstituted collagen preparations, however, it is exceedingly difficult to make such a distinction due to the influence of processing and crosslinking, and the presence of noncollagenous impurities. For the purposes of the present discussion, the treatment of Crumpton[23] has been adopted: antigenicity will be used to refer exclusively to the ability to *interact* with secreted antibodies, while immunogenicity will be used to refer to the ability to *induce* an immune response—a process that includes the synthesis of (and interaction with) these same antibodies.

Antigenicity

In general, macromolecular features of a protein not common to the host species are more likely to encourage an immune response than shared features. Thus, the issue of collagen antigenicity is intimately linked with the concepts of self-tolerance and interspecies variation. Although reconstituted collagens derived from human amnion have been developed and applied to eliminate interspecies variation in preclinical models,[24,25] no such product has yet received approval for clinical use, and thus an understanding of the antigenicity of exogenous collagen remains an issue of utmost importance.

Macromolecular features on an antigen molecule that interact with antibodies are referred to as antigenic determinants, some of which elicit strong interactions, some weak interactions, and some no interactions at all. Antigenic determinants of collagen can be classified into one of three categories:

1. Helical [Fig. 1(a)]—recognition by antibodies dependent on 3D conformation (i.e., the presence of an intact triple helix)
2. Central [Fig. 1(b)]—located within the triple helical portion of native collagen, but recognition based solely on amino acid sequence and not 3D conformation
3. Terminal [Fig. 1(c)]—located in the nonhelical terminal regions (telopeptides) of the molecule

The triple helical region of collagen has shown a high degree of evolutionary stability, with variations in the amino acid sequences not exceeding more than a few percent between mammalian species.[26] A far greater degree of variability is found in the nonhelical terminal regions, with up to half of the amino acid residues in these regions exhibiting interspecies variation.[15] It is thus, perhaps, not surprising that a number of studies have shown that the major antigenic determinants for certain donor/recipient pairings are located within these terminal regions.[27–30] In contrast, however, studies performed using different species pairings have shown the major determinants to be helical,[31–33] and in still other cases, evidence has been presented to suggest that central determinants also play a major role in collagen–antibody interactions.[32] It is pertinent to note that central determinants are often hidden eptitopes, only interacting with antibodies when the triple helix has unwound;[34] this fact may have implications for the antibody response to collagenous implants as they denature of degrade.

Such variability clarifies an oft-encountered misconception, namely, that the majority of—or even *all*—collagen antigenicity is, without exception, attributable to its terminal telopeptides.[37–40] Although certain documented cases have indicated that this holds true for some donor/recipient pairings, a thorough examination of the literature indicates that the location of major antigenic determinants on the collagen molecule varies depending on both the donor and recipient species (Table I). Although one study has been performed on the helical and central determinants of bovine collagen in humans,[7] further detailed study is needed to characterize the human immune response.

Immunogenicity

The immune response to an antigen involves a number of molecule and cell types. Although binding of antibodies and targeting by cytotoxic cells represent the mechanisms through which antigens and antigen-infected cells are ultimately eliminated, the cascade of events linking exposure to elimination comprises a complex—and, at times, poorly understood—interaction between the humoral (antibody-mediated) and cell-mediated responses (Fig. 2)

The humoral response involves the production of immunoglobin (Ig) molecules (antibodies) that bind directly to antigens, blocking their active sites and marking them for destruction by phagocytes and natural killer cells. In contrast, the cell-mediated response involves cell types that do not interact directly with antigens (T-cells), but interact instead with host cells that show signs either of (1) being infected with antigens or (2) having engulged them through phagocytosis. Far from acting independently, however, these two response mechanisms interact in certain instances, with antibody-producing B-cells functioning only under the regulatory influence of T-cells in some cases (T-cell–dependent humoral response) and independently in others (T-cell–independent humoral response). Similarly, cell-mediated responses to multicellular organisms too large to be phagocytosed are often dependent on the prior attachment of antibodies.

In some individuals or species, immunological responses to certain antigens are absent (immunological tolerance), while in others a predisposition to strong responses to a given antigen exists (hypersensitivity or allergy). Although both of these conditions are often genetic, tolerance and hypersensitivity can also be acquired after repeated or heavy exposure. Disease and other altered immunological conditions can alter the immune response to a given antigen, and, in extreme cases, autoimmune disorders can develop, in which adverse responses to the body's own tissue occur.

The immune response to collagen contains both a humoral and a cell-mediated component, the relative contributions of which are not yet fully understood. Experiments in a murine model have shown that the humoral response to bovine collagen is T-cell dependent, with no measurable antibody re-

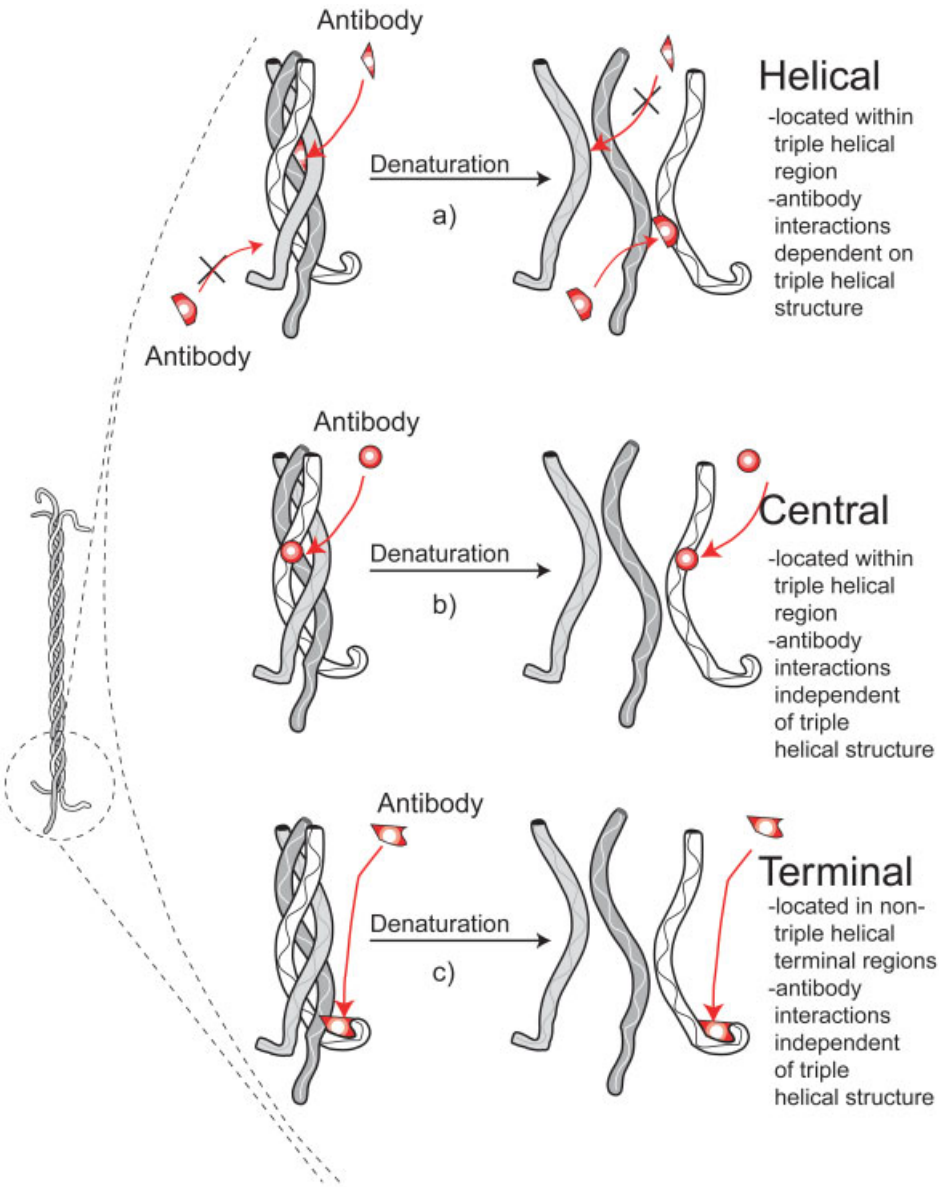

Figure 1. Classes of antigenic determinants of collagen.[15] [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

sponse in the absence of T-cells.[41] However, murine reactions to rat and Ascaris collagens have been shown to be T-cell independent,[42] illustrating that—as in the case of antigenicity—the immunogenic response elicited by collagen is dependent both on the donor and the recipient species.

Exposure to exogenous collagen is believed to be primarily dietary in nature. This is in contrast to airborne allergens such as pollen, and to contact allergens such as latex and nickel. Clinical observations indicate that 2–4% of the total population possess an inherent immunity (allergy) to bovine

TABLE I. Species Dependence of the Antibody Response to Type I Collagen[15]

Donor Species	Recipient Species	Major Antigenic Sites	Minor Antigenic Sites	Sites Apparently Not Involved	Ref
Calf	Rabbit	Terminal	Helical, Central	—	30
Rat	Rabbit	Terminal	Helical, Central	—	29
Rat	Chicken	Helical, Central	—	Terminal	31,32
Calf	Rat	Helical	—	Terminal, Central	31,35,36
Calf	Mouse	Helical	—	Terminal, Central	33

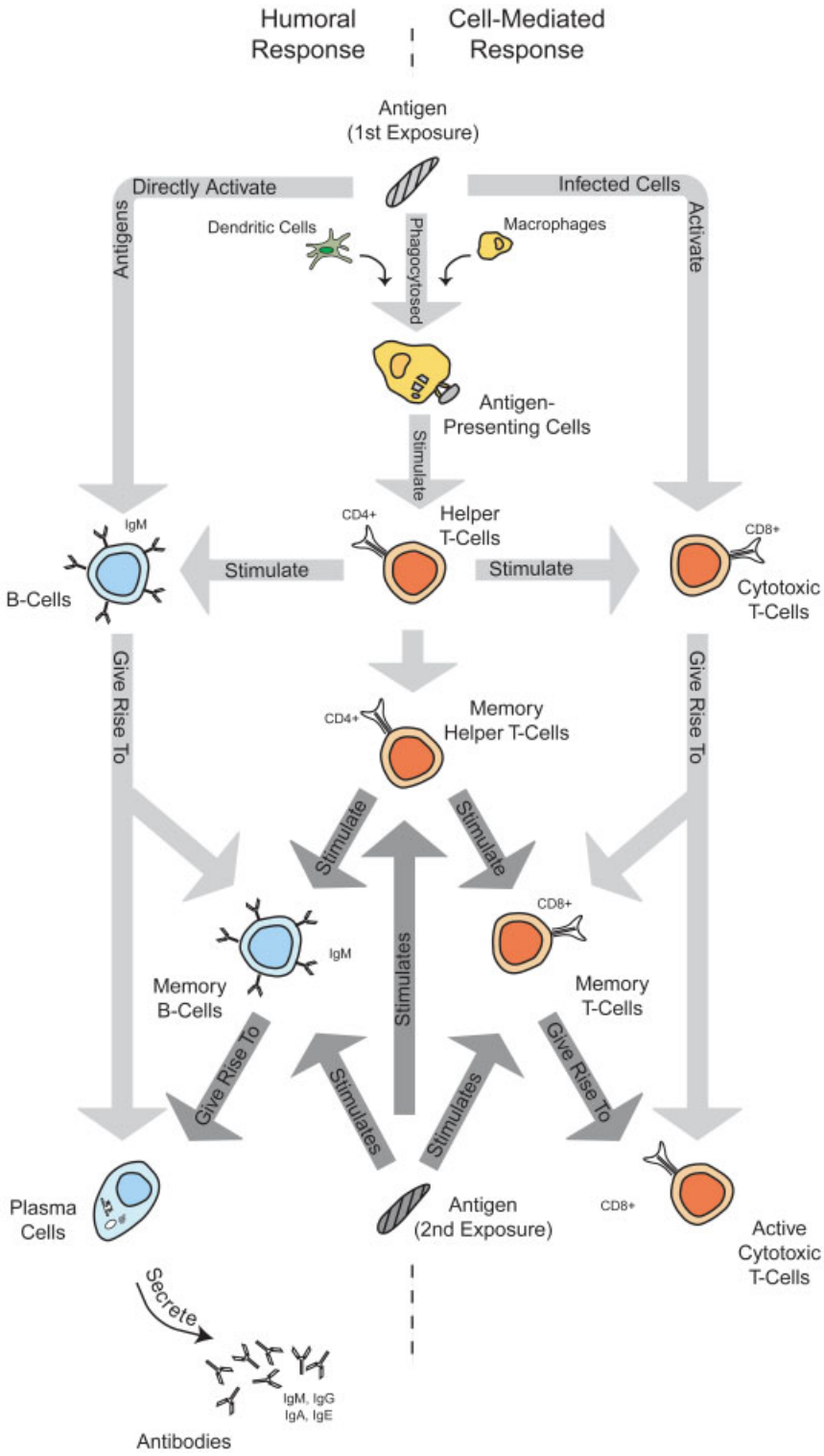

Figure 2. Humoral and cell-mediated immune responses. IgM—immunoglobin (Ig) molecule acting as the antigen receptor on the surface of B-cells (also the first antibody secreted during primary response); IgG—predominant serum Ig, appears after initial secretion of IgM; IgA, IgE—serum antibodies also appearing after initial secretion of IgM; $CD4^+$—surface marker/molecule characterics tic of helper T-cells; $CD8^+$—surface marker/molecule characteristic of cytotoxic T-cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

type I collagen.[43–46] Comparison with the 10–15% of the total population susceptible to nickel-sensitization,[47,48] or the estimated 6% (7–17% of healthcare workers) susceptible to latex allergy[49] shows this incidence to be decidedly low.

Common clinical practice assesses the risk of immune reactions to collagen on the basis of levels of circulating antibodies, with most physicians recommending two skin tests prior to treatment.[50] Although experience suggests that these precautions greatly reduce the number patients developing immune reactions, an additional 1–2% of patients still encounter them.

CLINICAL RESPONSES TO COLLAGEN

The first widespread use of collagen in the surgical environment was in the capacity of the suture material most commonly referred to as catgut.[51] Catgut consists of intestinal tissue from cattle or sheep treated to remove all noncollagenous material, and crosslinked using a variety of chemical treatments;[52] there has been no known use of the intestines of a cat. Although a number of studies inferring possible allergic reactions were documented from 1930–1970,[53,54] an equal number of studies argued against such a relationship, attributing the stigma of catgut allergy to septic complications or psychosomatic factors.[52,55] Although precise immunochemical studies using human antibodies would undoubtedly contribute towards elucidating the questions regarding catgut allergy, the advent of synthetic polymer sutures has made their tissue-derived equivalents somewhat obsolete, and hence the likelihood of such a study small.

Perhaps the most thoroughly characterized collagen-based devices are the injectable collagens used for soft tissue augmentation. Generally produced via pepsin extraction from calf skin, these products are used commonly in cosmetic surgery, and immunological studies documenting responses to a number of them provide a useful tool for the statistical evaluation of immune responses to collagen in general.[43–45,56,57] Results from these studies have consistently shown the incidence of preexisting hypersensitivity to bovine collagen to be in the range of 2–4% percent, with the postoperative development of bovine collagen allergy in an additional 1% of subjects.[43–45] In the rare (<3%) incidence of adverse reactions, granuloma and localized inflammation have been observed—reactions that generally resolve within a few months, and never last longer than 1 year. Premature resorption or other adverse effects on implant function have not been reported in conjunction with these reactions, and their treatment using immunosuppressants has been shown to be effective.[6] Routine practice dictates that all patients are prescreened for preexisting collagen allergy, with patients exhibiting signs of hypersensitivity excluded from treatment.

Composites of pepsin-solubilized bovine type I collagen and calcium phosphates have recently seen significant use as bone fillers for spinal fusion,[58,59] fracture fixation,[60,61] and maxillofacial applications.[62–64] As these materials are uncrosslinked, they provide little in the way of mechanical strength, but when used in combination with internal or external fixation devices they have produced encouraging results. Immunological responses to these materials have been limited to elevated levels of circulating antibodies to collagen, with no reported effect on the efficacy of the implant itself.[60,62,63] As with the case of injectable collagen, screening for collagen allergy is routinely performed prior to implantation.

Dermal substitutes for wound cover and wound closure have provided an application for some of the most advanced collagenous implants. These devices have generally been layered to mimic the histological structure of skin, and have included, among others: (a) porous glutaraldehyde- and DHT-crosslinked collagen–glycosaminoglycan copolymers combined with a silicone membrane,[65–67] (b) trilayered assemblies of silicone, nylon mesh, and collagen,[68–70] and (c) neonatal keratinocytes alternated with neonatal fibroblasts seeded in collagen.[46,71]

Both bovine[67,71] and porcine[69] dermal collagens have been used to develop these products, with both acid extraction[67,71] and pepsin treatment[70] employed depending on the device. Although immunological data regarding the clinical use of these materials is arguably less comprehensive than that for other implant types, no collagen-induced adverse immunological responses to nonallograft dermal substitutes have been documented, despite the numerous collagen sources and varied extraction methods used (Table II). The absence of any documented variations in the human immunological response to devices produced from both acid-solubilized (telopeptide-intact) collagen and pepsin-solubilized (telopeptide-deficient) collagen is particularly noteworthy, as it suggests that telopeptide removal provides no immunological benefit of clinical significance.

The ability of fibrillar collagen to promote platelet aggregation and subsequent clotting has led to the use of collagen-containing devices as hemostats,[72–75] particularly in applications where blood vessels cannot easily be clamped.[75,76] Although largely successful, their use has shown both marginally higher incidences of induced collagen allergy,[72] and a granulomatous foreign-body reaction was observed after application of a microfibrillar collagen hemostat in the spleen.[8] It should be noted, however, that such devices often contain noncollagenous protein contaminants, which some studies have been shown to be the main immunogenic components of collagen hemostats.[17,73]

Documented immunological reactions to other forms of collagen devices have generally followed the trends of dermal, osseous, and cosmetic devices, with adverse immune reactions occurring extremely infrequently. Fluid buildup (oedema, angioedema) has been reported in the throat and periocular regions following both ingestion of bovine collagen and use of bovine collagen corneal shields,[3] but only in isolated cases without statistical data indicating incidence of occurrence.

TABLE II. Immunological Observations from Selected Clinical Trials

			Collagen			Documented Trials			
	Product	Composition	Type	Extraction	X-Linking	Applications	Cases	Immunological Responses	Ref
Cosmetic	Zyderm/Zyplast Collagen Corp, Palo Alto, CA	Collagen	Bovine dermal Type I (95%), Type III (5%)	Pepsin	None	Injectable soft tissue augmentation	>1,000,000	preexisting bovine collagen allergy in 2% of patients; 1% developed allergy in response to implant; adverse reaction to implant (localized inflammation, granuloma formation) in 1% of patients	43,44,77
	Atelocollagen Koken: Tokyo, Japan	Collagen	Bovine dermal Type I	Pepsin	None	Injectable soft tissue augmentation	705	preexisting bovine collagen allergy in 3.8% of patients; adverse reaction (localized inflammation) to implant observed in 2.3% of patients	45
Dermatologic	Integra Integra Life Science Corp, Plainsboro, NJ	Collagen-GAG/ silicone	Bovine dermal Type I	Acid	Glut, DHT	Skin substitute for wound closure	159	no adverse reactions; specific immunological data not presented	67,78–81
	Apligraf Organogenesis Inc., Canton, MA	Keratinocytes/ collagen and fibroblasts	Bovine dermal Type I	Acid	None	Skin substitute for wound cover	107	preexisting bovine collagen allergy in 3.0% of patients; no patients developed allergy in response to implant; no adverse reactions of any kind observed in response to grafts	46,71
Orthopaedic	Collagraft Zimmer Corporation, Warsaw, IN	Collagen/HAp/ TCP	Bovine dermal Type I	Pepsin	None	Bone filler for spinal fusion, fracture fixation	303	postoperative development of bovine collagen allergy observed in 0.33% of patients (1 case); no associated complications	59,60,82
	Alveoform Collagen Corporation, Palo Alto, CA	Collagen/HAp	Bovine dermal Type I	Not specified	Not specified	Bone filler for maxillae and mandibular augmentation	77	preexisting bovine collagen allergy in 6.5% of patients; additional 6.5% developed allergy postoperatively; no adverse affect on surgical outcome	62,63
Other	CoStasis Cohesion Technologies, Palo Alto, CA	Collagen/thrombin	Bovine dermal Type I	Pepsin	None	Sprayable surgical hemostat	92	preexisting bovine collagen allergy in 1% of patients; additional 8% developed allergy in response to implant; no adverse affects on operative outcome	72
	Nerve Regeneration Conduit Noncommercial, Kyoto, Japan	PGA tube/collagen filler	Porcine dermal Type I (85%), Type III (15%)	Pepsin	DHT	Digital, peroneal nerve grafting	65	no adverse reactions; specific immunological data not presented	83,84

GAG = Glycosaminoglycan; HAp = Hydroxyapatite; TCP = tricalcium phospate; Glut = glutaraldehyde; DHT = dehydrothermal treatment

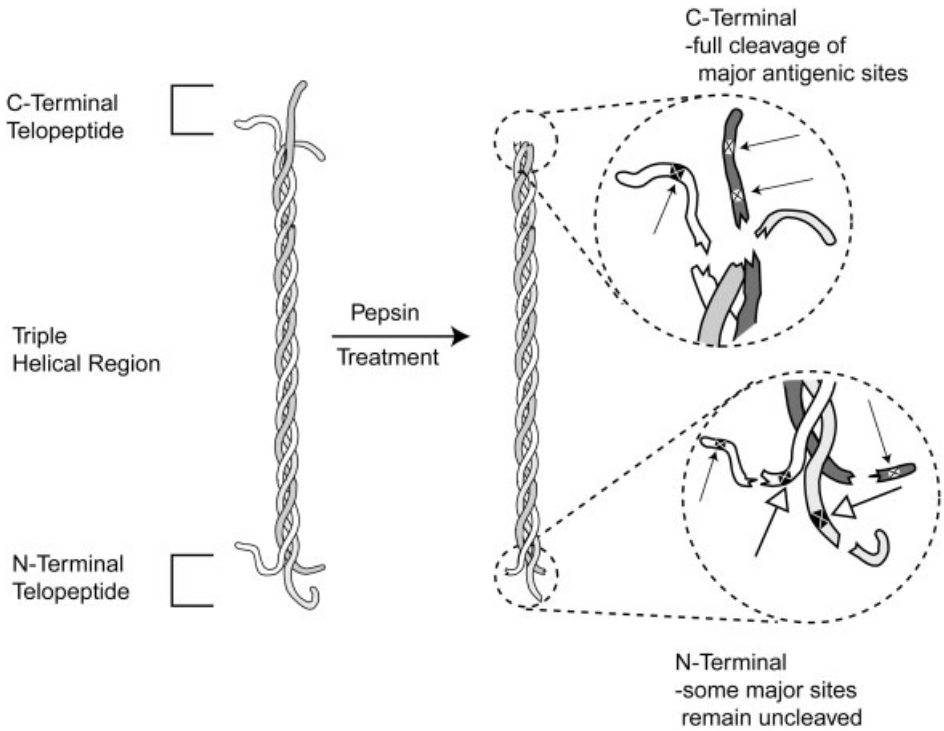

Figure 3. Telopeptide removal via pepsin treatment.

RECENT ISSUES

Atelocollagen

Over the past decade, the term atelocollagen—not to be confused with the commercial dermal substitute of the same name (Atelocollagen®)[38]—has been used with increasing frequency to refer to collagens treated with proteolytic enzymes to remove the terminal telopeptides.[38,85–88] The appearance of this term did not actually correspond to the development of any new extraction method, but was rather a means to underscore the purported immunological benefits of telopeptide removal.

Telopeptide cleavage results in collagen whose triple-helical conformation is intact,[28,30,39] yet as both the amino (N)- and carboxyl (C)-telopeptides play important roles in crosslinking and fibril formation, their complete removal results in an amorphous arrangement of collagen molecules and a consequent loss of the banded-fibril pattern in the reconstituted product.[90] Furthermore, telopeptide removal results in a significant increase in solubility.

The original observations that telopeptide cleavage affects the antigenic response to collagen were made following pepsin treatment,[27,28,30,89] and it is thus not surprising that pepsin is the most commonly used enzyme for producing implant-grade atelocollagen. Although it is well established that pepsin cleaves only at sites within the terminal regions, a common misconception is that it completely removes both the N- and C-telopeptides. In fact, it has been shown that, in some cases, telopeptide remnants persisting following pepsin treatment (Fig. 3) are sufficiently large that the antigenic activity of the pepsin-treated and native forms are largely indistinguishable.[31] The ability of pepsin-solubilized collagen to form fibrils upon coprecipitation with calcium salts[91] may also be due in part to the fibril-forming capacity imparted by residual telopeptides.

As discussed previously, the location of the major antigenic sites on the collagen molecule varies depending on donor/recipient species pairing. The lone documented investigation of the human antigenic response to exogenous collagen reported the helical and central antigenic determinants of pepsin-solubilized (telopeptide-deficient) bovine collagen against human antiserum;[7] no such studies of the terminal antigenic sites on bovine collagen or investigations of any of the antigenic sites on collagen from other species have been documented. Thus, direct evidence showing that the major antigenic determinants for the bovine/human and porcine/human donor/recipient pairs reside within the telopeptides has yet to be produced.

Numerous publications[37–39] and even a long-standing patent[40] claim that telopeptide removal results in collagen that is "nonimmunogenic, or possesses a negligibly low level of immunogenicity."[40] These claims are often supported by selective referencing of studies performed on calf/rabbit[28,30] and rat/rabbit[29] donor/recipient pairs. This practice is, however, grossly misleading, as it ignores the wealth of evidence showing the major antigenic determinants of other donor/recipient pairs to be central or helical, with terminal sites having no apparent involvement in the antibody response whatsoever.[31–33]

Given the high degree of interspecies variation within the telopeptides,[15] it is difficult to argue against the existence of

at least a *potential* immunological benefit of using telopeptide-depleted collagen in place of acid soluble forms, and indeed, there may exist a number of processing-related benefits resulting from the increased solubility and amorphous nature of atelocollagen. At present, however, the claim that it provides any clinically significant immunological benefit remains unsubstantiated.

Crosslinking

Considerable literature has been devoted to the development of crosslinking treatments to tailor the mechanical and degradation properties of collagen implants. Physical,[92–95] chemical,[21,96–99] and combination[67,87,100] treatments have been extensively developed, characterized, and applied in both *in vitro* and *in vivo* models.

Of these methods, glutaraldehyde treatment is by far the most well known and well characterized, and is still the only commercially viable process to have achieved widespread acceptance.[99] Despite its many advantages, however, much recent literature has been devoted to the development of alternative crosslinking treatments, to avoid the well-documented cytotoxic reactions that glutaraldehyde is known to have the potential to evoke.[20,21,101–103] It should be noted, however, that although these reactions have been reported often *in vitro*, evidence of their occurrence has yet to be produced *in vivo*. In any event, cytotoxic reactions to glutaraldehyde result from the persistence of residual traces of the crosslinking agent itself, and not from changes in the structure of collagen.

Glutaraldehyde treatment has long been believed to reduce the antigenicity of xenogenic collagen.[22] This claim has often been supported by arguments that crosslink formation shields or modifies major antigenic sites, thus reducing their capacity to interact with antibodies. Although such arguments are plausible, they have not been substantiated by clinical and immunological evidence, and recent evidence—obtained following the implantation of xenogenic caprine heart valves in a canine model—has, in fact, shown glutaraldehyde treatment to result in *increased* antigenicity.[104]

In general, the changes in immunochemical behavior induced by glutaraldehyde—and indeed all crosslinking techniques—are as yet poorly understood, and thus explicit experimental evidence is required to determine the immunological effects of a given crosslinking treatment.

Dependence of Immunogenicity on Implantation Site

The discussion of antigenicity to this point has focused mainly on devices containing isolated and purified collagen in soluble or solubilized form. However, the use of decellularized sections of extracellular matrix for tissue regeneration scaffolds is a well-known and well-studied technique, and an examination of studies pertaining to their immunological properties reveals a pertinent feature. It should be noted that such grafts contain, in addition to collagen, antigenic noncollagenous proteins,[17] and—depending on the method of decellularization—residual cell-associated components,[104] whose presence or absence typically dominates observed immunogenic responses.[105]

Despite the demonstrated immunogenicity of porcine and bovine xenograft heart valves,[18,104] their clinical application has shown that they do not generally illicit an adverse reaction.[11,12,106] It has been proposed that this apparent immunosilence is due primarily to the fact that the high flow environment of the aortic outflow tract shields the grafts from cellular interactions,[104] or, stated more generally, isolates them from the lymphatic system as a whole. Support for this hypothesis has been provided in the form of host-versus-graft type rejection of a commonly-used heart valve material, observed upon intramuscular implantation—an implantation site that provides ready exposure to the immune system.[20]

Studies of the immune responses evoked at various osseous implantation sites have similarly shown that marrow exposure at cancellous sites results in foreign-body reactions both more sensitive and more reproducible than responses evoked at cortical sites, which physically are more isolated from the marrow and its source of lymphoid progenitor cells.[107] Furthermore, major thermal injury is known to activate an inflammatory cascade thought to contribute to the development of postburn immunosuppression.[108] Although dermal substitutes are not physically isolated from the lymphatic system following thermal injury, suppressed reactivity, expansion, and differentiation of T- and B-cells can mean that grafts are largely isolated from its activity.

Such evidence demonstrates the importance of careful selection of preclinical models for evaluating immune responses to new devices, as evaluation in shielded sites or under altered immunological conditions may not provide a full representation of the response elicited in a given clinical application.

Collagen-Induced Autoimmunity

The discovery that injections of both allogenous and exogenous type II collagen emulsified in Freund's adjuvant induced arthritis in rats,[109] primates,[110,111] and certain strains of mice[112] triggered concerns that an analogous response could occur in humans. These fears were further supported by subsequent correlation between observations that antibodies to type II collagen play a major role in the initiation of this reaction,[113,114] and the observed presence of type II antibodies in rheumatoid arthritis patients.[115–117]

Although the parallels between collagen-induced arthritis in lab animals and rheumatoid arthritis in humans are both numerous and strong, to assess the risk of autoimmunity induced by collagen implants, a number of clarifications are pertinent:

1. types II and XI are the only collagens that have been shown to be arthritogenic;[118] collagen types I and III do not induce autoimmune reactions;[109,112]
2. induction of collagen-induced arthritis requires, at the very least, the presence of an adjuvant to amplify the immune response;[111,112]

3. unlike the case of collagen-induced arthritis, autoreactivity to cartilage type II collagen is not a defining feature of human rheumatoid arthritis, and may be a consequence of the disease as opposed its cause;[119]
4. reactions to collagen implants observed in humans to date have been directed exclusively at the implant itself, and have not in any way been autoimmune in nature.

Although there is no evidence to support the theory that induced autoimmunity in humans could result from the implantation of devices containing type II collagen, there is equally no direct evidence to disprove it. Although a number of type II collagen containing devices have been tested in animal models without adverse immune responses,[120,121] none of these studies have been performed in species previously shown to be susceptible to collagen-induced arthritis.

Because nearly all current collagen-containing implants are composed of type I (and to a lesser extent type III), collagen-induced autoimmunity is not generally considered a potential concern. However, with the progress of research to address the issue of repair and regeneration of cartilage—a tissue rich in type II collagen—is likely to come increased use of type II collagen as an implant material. Thus, the results of ongoing research aimed at elucidating the pathology of collagen-induced arthritis is, and will continue to be, of great interest.

CONCLUSIONS

The success of collagen as a biomaterial is due in no small part to its low antigenicity and immunogenicity. Nonetheless, for the design and application of new biomedical devices, an understanding of the underlying mechanisms of the human immune response to collagen—and the clinical significance thereof—is still of utmost importance. In light of the wealth of literature pertaining to collagen immunochemistry, care must be taken to ensure that the results of studies performed on specific donor/recipient species-pairs are not used to make broad generalizations regarding the immunological compatibility of all collagen types. Care should similarly be taken to ensure that claims that certain processing treatments reduce antigenicity are based on experimental evidence, and not merely on conjecture. Furthermore, due diligence is required to ensure that the risks posed by newly discovered disorders and pathogens are thoroughly assessed and addressed appropriately.

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REFERENCES

1. Li ST. Biologic biomaterials: Tissue-derived biomaterials (Collagen). In: Brozino JD, editor. The biomedical engineering handbook. Boca Raton, FL: CRC Press; 1995. p 627–647.
2. Gorham SD. Collagen. In: Byrom D, editor. Biomaterials. New York: Stockton Press; 1991. p 55–122.
3. Mullins RJ, Richards C, Walker T. Allergic reactions to oral, surgical and topical bovine collagen. Anaphylactic risk for surgeons. Aust N Z J Opthalmol 1996;24:257–260.
4. Rapaport M. Granuloma annulare caused by injectable collagen. Arch Dermatol 1984;120:837–838.
5. Webster RC, Kattner MD, Smith RC. Injectable collagen for augmentation of facial areas. Arch Otolaryngol 1984;110:652–656.
6. Baumann LS, Kerdel F. The treatment of bovine collagen allergy with cyclosporin. Dermatol Surg 1999;25:247–249.
7. Ellingsworth LR, DeLustro F, Brennan JE, Sawamura S, McPherson J. The human immune response to reconstituted bovine collagen. J Immunol 1986;136:877–882.
8. McGregor DH, MacArthur RI, Carter T. Avitene granulomas of colonic serosa. Ann Clin Lab Sci 1986;16:296–302.
9. Bauer TW, Muschler GF. Bone graft materials—An Overview of the basic science. Clin Orthop 2000;371:10–27.
10. Block JE, Poser J. Does xenogeneic demineralized bone-matrix have clinical utility as a bone-graft substitute. Med Hypotheses 1995;45:27–32.
11. Park SZ, Reardon MJ. Current status of stentless aortic xenografts. Curr Opin Cardiol 2000;15:74–81.
12. Reardon MJ, David TE. Stentless xenograft aortic valves. Curr Opin Cardiol 1999;14:84–89.
13. Yannas IV. Collagen and gelatin in the solid state. J Macromol Sci Rev Macromol Chem 1972;C7:49–104.
14. Friess W. Collagen—Biomaterial for drug delivery. Eur J Pharmaceut Biopharmaceut 1998;45:113–136.
15. Furthmayr F, Timpl R. Immunochemistry of collagens and procollagens. Int Rev Connect Tissue Res 1976;7:61–99.
16. Timpl R. Immunological studies on collagen. In: Ramachandran GN, Reddi AH, editors. Biochemistry of collagen. New York: Plenum Press; 1976.
17. DeLustro F, Condell RA, Nguyen MA, McPherson JM. A comparative-study of the biologic and immunological response to medical devices derived from dental collagen. J Biomed Mater Res 1986;20:109–120.
18. Allaire E, Guettier C, Bruneval P, Plissonnier D, Michel JB. Cell-free arterial grafts: Morphologic characteristics of aortic isografts, allografts, and xenografts in rats. J Vasc Surg 1994; 19:446–456.
19. Esses SI, Halloran PF. Donor marrow-derived cells as immunogens and targets for the immune-response to bone and skin allografts. Transplantation 1983;35:169–174.
20. Dahm M, Lyman WD, Schwell AB, Factor SM, Frater RW. Immunogenicity of glutaraldehyde-tanned bovine pericardium. J Thorac Cardiovasc Surg 1990;99:1082–1090.
21. Jayakrishnan A, Jameela SR. Glutaraldehyde as a fixative in bioprostheses and drug delivery matrices. Biomaterials 1996; 17:471–484.
22. Speer DP, Chvapil M, Eskelson CD, Ulreich J. Biological effects of residual glutaraldehyde in glutaraldehyde-tanned collagen biomaterials. J Biomed Mater Res 1980;14:753–764.
23. Crumpton MJ. Protein antigens: The molecular basis of antigenicity and immunogenicity. In: Sela M, editor. The antigens. New York: Academic Press; 1974. p 1–78.
24. Spira M, Liu BC, Xu ZL, Harrell R, Chahadeh H. Human amnion collagen for soft-tissue augmentation—Biochemical characterizations and animal observations. J Biomed Mater Res 1994;28:91–96.
25. Liu B, Harrell R, Xu ZL, Dresden MH, Spilker M. Immune response to gamma-irradiated injectable human amnion and human skin collagens in the rat. Arch Dermatol 1989;125: 1084–1089.
26. Fietzek PP, Kuhn K. The primary structure of collagen. Int Rev Connect Tissue Res 1976;7:1–60.

27. Furthmayr F, Beil W, Timpl R. Different antigenic determinants in the polypeptide chains of human collagen. FEBS Lett 1971;12:341–344.
28. Davison PF, Levine L, Drake MP, Rubin A, Bump S. The serologic specificity of tropocollagen telopeptides. J Exp Med 1967;126:331–349.
29. Michaeli D, Martin GR, Kettman J, Benjamin E, Leung DYK, Blatt BA. Localization of antigenic determinants in the polypeptide chains of collagen. Science 1969;166:1522–1524.
30. Pontz B, Meigel W, Rauterberg J, Kuhn K. Localization of two species specific antigenic determinants on the peptide chains of calf skin collagen. Eur J Biochem 1970;16:50–54.
31. Beil W, Timpl R, Furthmayr F. Conformation dependence of antigenic determinants on the collagen molecule. Immunology 1973;24:13–24.
32. Furthmayr F, Stoltz M, Becker U, Beil W, Timpl R. Chicken antibodies to soluble rat collagen. II. Specificity of the reactions with individual polypeptide chains and cyanogen bromide peptides of collagen. Immunochemistry 1972;9:789–798.
33. Nowack H, Hahn E, Timpl R. Specificity of the antibody response in inbred mice to bovine type I and type II collagen. Immunology 1975;29:621–628.
34. Dodge GR, Poole AR. Immunohistochemical detection and immunochemical analysis of type-II collagen degradation in human normal, rheumatoid, and osteoarthritic articular cartilages and in explants of bovine articular-cartilage cultured with interleukin-1. J Clin Invest 1989;83:647–661.
35. Hahn E, Timpl R. Involvement of more than a single polypeptide chain in the helical antigenic determinants of collagen. Eur J Immunol 1973;3:442–446.
36. Hahn E, Timpl R, Miller EJ. The production of specific antibodies to native collagens with the chain compositions, (alpha1(I))3, (alpha1(II))3, and (alpha1(I))2alpha 2. J Immunol 1974;113:421–423.
37. Iwasa J, Ochi M, Uchio Y, Katsube K, Adachi N, Kawasaki K. Effect of cell density on proliferation and matrix synthesis of condrocytes embedded in atelocollagen gel. Artif Organs 2003;27:249–255.
38. Sakai D, Mochida J, Yamamoto Y, Nomura T, Okuma M, Nishimura K, Nakai T, Ando K, Hotta T. Transplantation of mesenchymal stem cells embedded in atelocollagen((R)) gel to the intervertebral disc: A potential therapeutic model for disc degeneration. Biomaterials 2003;24:3531–3541.
39. Uchio Y, Ochi M, Matsusaki M, Kurioka H, Katsube K. Human chondrocyte proliferation and matrix synthesis cultured in atelocollagen (R) gel. J Biomed Mater Res 2000;50: 138–143.
40. Luck EE, Daniels JR, inventors; Collagen Corporation, assignee. US4233360: Non-antigenic collagen and articles of manufacture. USA. 1980.
41. Nowack H, Hahn E, Timpl R. Requirement for T cells in the antibody response of mice to calf skin collagen. Immunology 1976;30:29–32.
42. Fuchs S, Mozes E, Maoz A, Sela M. Thymus independence of a collagen-like synthetic polypeptide and of collagen, and the need for thymus and bone marrow-cell cooperation in the immune response to gelatin. J Exp Med 1974;139:148–158.
43. Cooperman L, Michaeli D. The immunogenicity of injectable collagen. 1. A 1-year prospective-study. J Am Acad Dermatol 1984;10:638–646.
44. Cooperman L, Michaeli D. The immunogenicity of injectable collagen. 2. A retrospective review of 72 tested and treated patients. J Am Acad Dermatol 1984;10:647–651.
45. Charriere G, Bejot M, Schnitzler L, Ville G, Hartmann DJ. Reactions to a bovine collagen implant—Clinical and immunological study in 705 patients. J Am Acad Dermatol 1989; 21:1203–1208.
46. Eaglstein WH, Alvarez OM, Auletta M, Leffel D, Rogers GS, Zitelli JA, Norris JEC, Thomas I, Irondo M, Fewkes J, Hardin-Young J, Duff RG, Sabolinski ML. Acute excisional wounds treated with a tissue-engineered skin (apligraf). Dermatol Surg 1999;25:195–201.
47. Budinger L, Hertl M. Immunological mechanisms in hypersensitivity reactions to metal ions: An overview. Allergy 2000; 55:108–115.
48. von Blomberg van der Flier M, van der Burg CK, Pos O. In vitro studies in nickel allergy: Diagnostic value of a dual parameter analysis. J Invest Dermatol 1987;88:362–368.
49. Meyer KK, Beezhold DH. Latex allergy: How safe are your gloves? Bull Am Coll Surg 1997;82:13–15.
50. Elson ML. The role of skin testing in the use of collagen injectable materials. J Dermatol Surg Oncol 1989;15:301–303.
51. Snyder CC. On the history of the suture. Plastic Reconstruct Surg 1976;58:401–406.
52. Carroll RE. Surgical catgut: The myth of allergy. J Hand Surg [Br] 1989;14B:218–220.
53. Tripp HD. Catgut allergy (case report). J Ind State M Assoc 1935;28:383–384.
54. Getzen LC, Jansen GA. Correlation between allergy to suture material and postoperative wound infections. Surgery 1966; 60:824–826.
55. Sykes B. The molecular genetics of collagen. Bioessays 1985; 3:112–117.
56. Lemperle G, Hazangauthier N, Lemperle M. PMMA microspheres (artecoll) for skin and soft-tissue augmentation. 2. Clinical investigations. Plastic Reconstruct Surg 1995;96:627–634.
57. Millikan L. Long-term safety and efficacy with fibrel in the treatment of cutaneous scars—Results of a multicenter study. J Dermatol Surg Oncol 1989;15:837–842.
58. Burkus JK, Heim SE, Gornet MF, Zdeblick TA. Is INFUSE bone graft superior to autograft bone? An integrated analysis of clinical trials using the LT-CAGE lumbar tapered fusion device. J Spinal Disord Technol 2003;16:113–122.
59. Muschler GF, Negami S, Hyodo A, Gaisser D, Easley K, Kambic H. Evaluation of collagen ceramic composite graft materials in a spinal fusion model. Clin Orthop1996;328:250–260.
60. Cornell CN, Lane JM, Chapman M, Merkow R, Seligson D, Henry S, Gustillo R, Vincent K. Multicenter trial of collagraft as bone graft substitute. J Orthopaed Trauma 1991;5:1–8.
61. Chapman MW, Bucholz R, Cornell C. Treatment of acute fractures with a collagen-calcium phosphate graft material—A randomized clinical trial. J Bone Joint Surg [Am] 1997;79A: 495–502.
62. Mehlisch DR. Collagen/hydroxylapatite implant for augmenting deficient alveolar ridges: A 24-month clinical and histologic summary. Oral Surg Oral Med Oral Pathol 1989;68:505–514.
63. Mehlisch DR, Taylor TD, Leibold DG, Hiatt R, Waite DE, Waite PD, Laskin DM, Smith ST. Collagen/hydroxylapatite implant for augmentating deficient alveolar ridges. J Oral Maxillofac Surg 1988;44:839–843.
64. Hemmerle J, Leize M, Voegel JC. Long-term behavior of a hydroxyapatite collagen-glycosaminoglycan biomaterial used for oral-surgery—A case-report. J Mater Sci Mater Med 1995; 6:360–366.
65. Yannas IV, Burke JF, Orgill DP, Skrabut EM. Wound tissue can utilize a polymeric template to synthesize a functional extension of skin. Science 1982;215:174–176.
66. Burke JF, Yannas IV, Quinby WC, Bondoc CC, Jung WK. Successful use of a physiologically acceptable artificial skin in the treatment of extensive burn injury. Ann Surg 1981;194: 413–428.

67. Yannas IV, Lee E, Orgill DP, Skrabut EM, Murphy GF. Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin. Proc Natl Acad Sci USA 1989;86:933–937.
68. Purdue GF, Hunt JL, Gillespie RW, Hansbrough JF, Dominic WJ, Robson MC, Smith DJ, Macmillan BG, Waymac JP, Herndon DN, Desai M, Terry BE, Bendlin A, Declement FA, Kahn AM, Hanumadass ML, Matsuda T. Biosynthetic skin substitute versus frozen human cadaver allograft for temporary coverage of excised burn wounds. J Trauma Injury Infect Crit Care 1987;27:155–157.
69. Tavis MJ, Thornton JW, Bartlett RH, Roth JC, Woodroof EA. A new composite skin prosthesis. Burns 1980;7:123–130.
70. Boyce S, inventor The regents of the University of California, assignee. US5273900: Method and apparatus for preparing composite skin replacement. USA. 1993.
71. Eaglstein WH, Falanga V. Tissue engineering and the development of apligraf(R), a human skin equivalent. Clin Ther 1997;19:894–905.
72. Nelson PA, Powers JN, Estridge TD, Elder EA, Alea AD, Sidhu PK, Sehl LC, DeLustro FA. Serological analysis of patients treated with a new surgical hemostat containing bovine proteins and autologous plasma. J Biomed Mater Res 2001;58:710–719.
73. Tsuda H, Higashi S, Iwanaga S, Kubota T, Morita T, Yanaga K. Development of antitissue factor antibodies in patients after liver surgery. Blood 1993;82:96–102.
74. Browder IW, Litwin MS. Use of absorbable collagen for hemostasis in general surgical patients. Am Surg 1986;52: 492–494.
75. Sakon M, Monden M, Gotoh M, Kobayashi K, Kambayashi J, Mori T, Okamura J. Use of microcrystalline collagen powder and fibrinogen tissue adhesive for hemostasis and prevention of rebleeding in patients with hepatocellular-carcinoma associated with cirrhosis of the liver. Surg Gynecol Obstet 1989; 168:453–454.
76. Alexander J, Rabinowitz J. Microfibrillar collagen (Avitene) as a hemostatic agent in experimental oral wounds. J Oral Surg 1978;36:202–205.
77. Kaplan EN. Clinical variations in the utilization of zyderm-I and zyderm-Ii. Plastic Reconstuct Surg 1984;73:329.
78. Heimbach D, Luterman A, Burke J, Cram A, Herndon D, Hunt J, Jordan M, McManus W, Solem L, Warden G, Zawacki B. Artificial dermis for major burns—A multi-center randomized clinical-trial. Ann Surg 1988;208:313–320.
79. Michaeli D, MacPherson M. Immunologic study of artificial skin used in the treatment of thermal injuries. Burn Care Rehab 1990;11:21–26.
80. Yannas IV, Burke JF. Design of an artificial skin. I. Basic design principles. J Biomed Mater Res 1980;14:65–81.
81. Yannas IV, Burke JF, Gordon PL, Huang C, Rubenstein RH. Design of an artificial skin. II. Control of chemical composition. J Biomed Mater Res 1980;14:107–132.
82. Walsh WR, Harrison J, Loefler A, Martin T, Van Sickle D, Brown MK, Sonnabend DH. Mechanical and histologic evaluation of collagraft in an ovine lumbar fusion model. Clin Orthop 2000;375:258–266.
83. Hagiwara A, Nakashima S, Itoh T, Sakakura C, Otsuji E, Yamagishi H, Okajima S, Kusuzaki K, Hase H, Kubo S, Soh J, Miki T, Toba T, Nakamura T, Shimizu, Y. Clinical application of PGA-tube for regeneration of intrapelvic nerves during extended surgery for intrapelvic recurrent rectal cancer. Gan To Kagaku Ryoho 2002;29:2202–2204.
84. Nakamura T. Clinical application of nerve conduits consisting of a polyglycolic acid (PGA)-collagen composite tube filled with collagen sponge. Connect Tissue 2003;35:53–57.
85. Onodera J, Saito A, George J, Iwasaki T, Ito H, Aso Y, Hamano T, Kanai A, Miyata T, Nagai Y. Application of atelocollagen solution for lacrimal duct occlusion. In: Lacrimal gland, tear film, and dry eye syndromes 3: Basic science and clinical relevance, parts A and B. Norwell, MA: Kluwer Academic; 2002. p. 1277–1281.
86. Kohmura E, Yuguchi T, Yoshimine T, Fujinaka T, Koseki N, Sano A, Kishino A, Nakayama C, Sakaki T, Nonaka M, Takemoto O, Hayakawa T. BDNF atelocollagen mini-pellet accelerates facial nerve regeneration. Brain Res 1999;849: 235–238.
87. Vizarova K, Bakos D, Rehakova M, Macho V. Modification of layered atelocollagen by ultraviolet-irradiation and chemical cross-linking-structure stability and mechanical-properties. Biomaterials 1994;15:1082–1086.
88. Yamamoto S, Yoshimine T, Fujita T, Kuroda R, Irie T, Fujioka K, Hayakawa T. Protective effect of Ngf atelocollagen mini-pellet on the hippocampal delayed neuronal death in gerbils. Neurosci Lett 1992;141:161–165.
89. Schmitt FO, Lenvine L, Drake MP, Rubin AL, Pfahl O, Davison PF. The antigenicity of tropocollagen. Proc Natl Acad Sci USA 1964;51:493–497.
90. Kadler KE, Holmes DF, Trotter JA, Chapman JA. Collagen fibril formation. Biochem J 1996;316:1–11.
91. Kikuchi M, Itoh S, Ichinose S, Shinomiya K, Tanaka J. Self-organization mechanism in a bone-like hydroxyapatite/collagen nanocomposite synthesized in vitro and its biological reaction in vivo. Biomaterials 2001;22:1705–1711.
92. Liu B, Harrell R, Davis RH, Dresden MH, Spira M. The Effect of gamma irradiation on injectable human amnion collagen. J Biomed Mater Res 1989;23:833–844.
93. Weadock KS, Miller EJ, Bellincampi LD, Zawadsky JP, Dunn MG. Physical crosslinking of collagen fibers: Comparison of ultraviolet irradiation and dehydrothermal treatment. J Biomed Mater Res 1995;29):1373–1379.
94. Weadock KS, Miller EJ, Keuffel EL, Dunn MG. Effect of physical crosslinking methods on collagen-fiber durability in proteolytic solutions. J Biomed Mater Res 1996;32:221–226.
95. Toba T, Nakamura T, Lynn AK, Matsumoto K, Fukuda S, Yoshitani M, Hori Y, Shimizu Y. Evaluation of peripheral nerve regeneration across an 80-mm gap using a polyglycolic acid (PGA)—Collagen nerve conduit filled with laminin-soaked collagen sponge in dogs. Int J Artif Organs 2002;25: 230–237.
96. Grabarek Z, Gergely J. Zero-length crosslinking procedure with the use of active esters. Anal Biochem 1990;185:131–135.
97. Girton TS, Oegema TR, Tranquillo RT. Exploiting glycation to stiffen and strengthen tissue equivalents for tissue engineering. J Biomed Mater Res 1999;46:87–92.
98. Kikuchi M, Taguchi T, Matsumoto HN, Takakuda K, Tanaka J. Cross-linkage of hydroxyapatite/collagen nano-composite with 3 different reagents. Bioceramics 2002;14:449–452.
99. Khor E. Methods for the treatment of collagenous tissues for bioprostheses. Biomaterials 1997;18:95–105.
100. Ohan MP, Weadock KS, Dunn MG. Synergistic effects of glucose and ultraviolet irradiation on the physical properties of collagen. J Biomed Mater Res 2002;60:384–391.
101. Huang-Lee LL, Cheung DT, Nimni ME. Biochemical changes and cytotoxicity associated with the degradation of polymeric glutaraldehyde derived crosslinks. J Biomed Mater Res 1990; 24:1185–1201.
102. van Luyn MJ, van Wachem PB, Olde Damink LH, Dijkstra PJ, Feijen J, Nieuwenhuis P. Secondary Cytotoxicity of cross-linked dermal sheep collagens during repeated exposure to human fibroblasts. Biomaterials 1992;13:1017–1024.
103. Yao CH, Sun JS, Lin FH, Liao CJ, Huang CW. Biological Effects and cytotoxicity of tricalcium phosphate and formaldehyde cross-linked gelatin composite. Mater Chem Phys 1996;45:6–14.

104. Courtman DW, Errett BF, Wilson GJ. The Role of crosslinking in modification of the immune response elicited against xenogenic vascular acellular matrices. J Biomed Mater Res 2001; 55:576–586.
105. DeLustro F, Dasch J, Keefe J, Ellingsworth L. Immune responses to allogeneic and xenogeneic implants of collagen and collagen derivatives. Clin Orthop 1990;260:263–279.
106. Cohen LH, Koster JK, Mee RB, Collins JJJ. Long-term follow-up of the Hancock bioprosthetic heart valve: A 6-year review. Circulation 1979;60:87–92.
107. Lu JX, Gallur A, Flautre B, Anselme K, Descamps M, Thierry B, Hardouin P. Comparative study of tissue reactions to calcium phosphate ceramics among cancellous, cortical, and medullar bone sites in rabbits. J Biomed Mater Res 1998;42:357–367.
108. Schwacha MG, Chaudry IH. The cellular basis of post-burn immunosuppression: Macrophages and mediators (review). Int J Mol Med 2002;10:239–243.
109. Trentham DE, Townes AS, Kang AH. Autoimmunity to Type II collagen: An experimental model of arthritis. J Exp Med 1977;146:857–868.
110. Yoo TJ, Stuart JM, Takeda T, Sudo N, Floyd RA, Ishibe T, Olson G, Orchik D, Shea JJ, Kang AH. Induction of type-I: I. Collagen autoimmune arthritis and ear disease in monkey. Ann N Y Acad Sci 1986;475:341–342.
111. Cathcart ES, Hayes KC, Gonnerman WA, Lazzari AA, Franzblau C. Experimental arthritis in a nonhuman primate:1. Induction by bovine type-II collagen. Lab Invest 1986;54:26–31.
112. Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B. Immunisation against heterologous type II collagen induces arthritis in mice. Nature 1980;283:666–668.
113. Svensson L, Jirholt J, Holmdahl R, Jansson L. B Cell-deficient mice do not develop type II collagen-induced arthritis (CIA). Clin Exp Immunol 1998;111:521–526.
114. Terato K, Hasty KA, Reife RA, Cremer MA, Kang AH, Stuart JM. Induction of arthritis with monoclonal-antibodies to collagen. J Immunol 1992;148:2103–2108.
115. Cook AD, Rowley MJ, Mackay IR, Gough A, Emery P. Antibodies to type II collagen in early rheumatoid arthritis—Correlation with disease progression. Arthritis Rheum 1996; 39:1720–1727.
116. Ronnelid J, Lysholm J, Engstromlaurent A, Klareskog L, Heyman B. Local anti-type-II collagen antibody-production in rheumatoid-arthritis synovial-fluid—Evidence for an HLA-DR4-restricted IgG response. Arthritis Rheum 1994;37:1023–1029.
117. Morgan K, Clague RB, Collins I, Ayad S, Phinn SD, Holt PJL. A longitudinal-study of anticollagen antibodies in patients with rheumatoid-arthritis. Arthritis Rheum 1989;32:139–145.
118. Cremer MA, Rosloniec EF, Kang AH. The cartilage collagens: A Review of their structure, organization, and role in the pathogenesis of experimental arthritis in animals and in human rheumatic disease. J Mol Med 1998;76:275–288.
119. Luross JA, Williams NA. The genetic and immunopathological processes underlying collagen-induced arthritis. Immunology 2001;103:407–416.
120. Mainil-Varlet P, Rieser F, Grogan S, Mueller W, Saager C, Jakob RP. Articular cartilage repair using a tissue-engineered cartilage-like implant: An animal study. Osteoarthritis Cartilage 2001;9:S6–S15.
121. Nehrer S, Breinan HA, Ramappa A, Hsu HP, Minas T, Shortkroff S, Sledge CB, Yannas IV, Spector M. Chondrocyte-seeded collagen matrices implanted in a chondral defect in a canine model. Biomaterials 1998;19:2313–2328.