A Morphological and Biochemical Analysis Comparative Study of the Collagen Products Biopad, Promogram, Puracol, and Colactive

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ABSTRACT

The aim of this study was to compare the capacity of the collagen products Biopad (Euroresearch, Milano, Italy), Promogran (Systagenix Wound Management, Quincy, Massachusetts), Colactive (Smith & Nephew, St Petersburg, Florida), and Puracol (Medline Industries, Mundelein, Illinois) to interact with biological tissues and to start restoring the healing process. These results demonstrate how these products can interact differently with enzymes and cells that characterize the environment of a healing wound.

KEYWORDS: Biopad, Promogram, Colactive, Puracol, collagen, comparative study of collagen products

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INTRODUCTION

Collagen, a major component of tendons, skin, cartilages, bones, and many other tissues, is a fibrous protein amounting to about 25% of total body protein content. The nature and handling characteristics of various collagen products can be determined in part by evaluating their susceptibility and their handling characteristics. In addition, to determine how collagen samples behave in vivo when placed on a healing wound or on skin with burns, collagen samples can be exposed in vitro to such cells as cultured fibroblasts (3T3), platelet-rich plasma, collagenase, or human lymphocytes (HLs).[1–4] Also, the use of chemical analysis, gel electrophoresis, electron microscopy, and cell culture provides several criteria by which collagen products can be studied and compared. Although the morphological analyses of the collagen products reported in this study are of a qualitative nature, the electrophoretic technique and the chemical analysis can be said to be of a quantitative nature because they allow the collagen contents and their respective polypeptide composition to be determined. Because fibroblasts play a key role in wound repair by producing such extracellular matrix components as different types of collagens and fibronectin, it is important to verify how these cell types can actually interact with collagen. In particular, it is essential to establish whether they can actually take contact with the 3-dimensional collagen matrix and whether they can move and proliferate freely under these conditions.[5,6] In contrast, leukocytes and lymphocytes have been shown to associate in vitro with collagen and its fragments as a crucial step in the initiation of the thrombus formation.[7] At the same time, lymphocytes are known to modulate the production of several growth factors so as to stimulate some immune reactions that may eventually lead to collagen degradation.[8]

In view of these roles, and perhaps for other and more complex reasons, it is essential to determine how lymphocytes migrate in a 3-dimensional collagen matrix, and whether they simply proceed by pseudopodia extension or by adhesive interactions.[3] Under these conditions, one can actually verify how collagen products become structurally modified and how long they are capable of persisting invariant before becoming fully disaggregated and absorbed by the host tissue. Different collagen products may differ in their propensity to undergo degradation, depending on the density of the mesh areas enclosed by collagen strands and on the extent they either shrink or swell upon exposure to proteolytic enzymes or macrophagic cells.

A comparison of Biopad (Euroresearch, Milano, Italy), Promogran (Systagenix Wound Management, Quincy, Massachusetts), Colactive (Smith & Nephew, St Petersburg, Florida), and Puracol (Medline Industries, Mundelein, Illinois) is summarized in Table 1. Biopad (represented as Condress [European brand name] in Figures) is a sterile lyophilized 100% type I native heterologous equine collagen that transforms into a soft gel, allowing contact with the entire wound bed. It is a primary wound dressing for topical use to control minor bleeding and for

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Table 1.

BIOPAD, PROMOGRAN, COLACTIVE, AND PURACOL COMPARISON

	Composition	Shape	Surface	Collagen Content	Porosity
Biopad	Lyophilized 100% type I native heterologous equine collagen	Square	25 cm^2	100%	217%
Promogran	Freeze-dried matrix composed of bovine collagen and oxidized regenerated cellulose	Hexagon	28.27 cm^2	57.20%	161%
Colactive	Porcine gelatin collagen and sodium alginate dressing that contains 90% porcine-derived collagen and 10% sodium alginate	Square	26.5 cm^2	83%	55.80%
Puracol	100% Pure native bovine-derived collagen dressing in its native, triple-helix format	Square	25 cm^2	88.40%	80%

wound management of any type of ulcer or skin lesion to help assist in wound closure. In a review of collagen and collagen-based wound dressings, it was the authors' opinion that when applied to a wound, Biopad constitutes a barrier for wound management against exogenous infective agents.[8,9] Promogran consists of a sterile, freeze-dried matrix composed of bovine collagen and oxidized regenerated cellulose, formed into an approximately 3-mm-thick sheet cut into hexagonal pieces. In the presence of wound exudate, the matrix absorbs liquid and forms a soft, conformable, biodegradable gel that physically binds and inactivates matrix metalloproteases, which have a detrimental effect on wound healing when present in excessive quantities.[9,10] A collagen dressing applied to a wound should absorb the hematic and lymphatic fluids present in the wound itself. From this point of view, the porosity of the product plays an important role. More porosity will allow greater absorption of the chronic wound fluid, thus providing an improved wound environment for healing. The gel also binds with naturally occurring growth factors within the wound and protects them from degradation by the proteases, releasing them back into the wound in an active form as the matrix is slowly broken down.

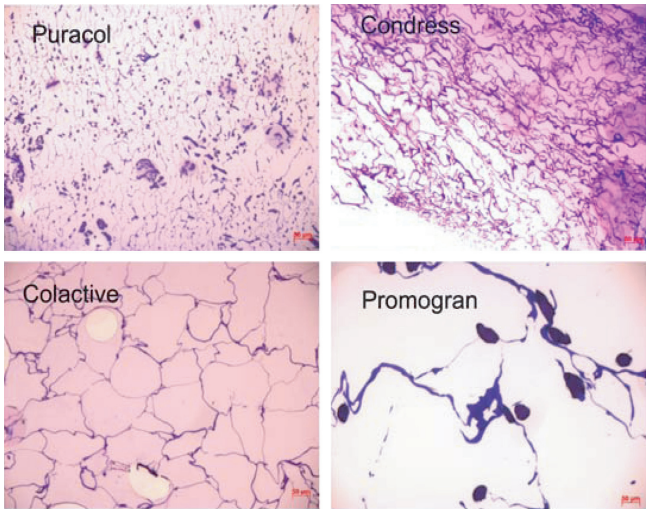
MATERIALS AND METHODS

The collagen products Biopad, Puracol, Promogran, and Colactive underwent chemical analysis to determine collagen content. To determine the dry weight of each collagen product, about 50 mg of each product was placed for 4 hours in a thermoregulated oven set at 105° C. Afterward, the collagen products were allowed to cool down in desiccators and eventually weighed. The nitrogen content was determined. By providing a direct reading of the nitrogen content for each collagen product, it was possible to obtain the actual collagen content through the 5.50 conversion factor from nitrogen to collagen. The hydroxyproline content in each collagen product was also calculated. When the nitrogen content was correlated with that of the dry matter, a determination of the actual value of collagen content can be made. The known values of the actual nitrogen and hydroxyproline content for each collagen product, together with their humidity content, allowed the authors to estimate the real content of dry collagen in each collagen product. To evaluate how much collagen is in contact with the wound surface, the extent of collagen distribution on the surface was considered. This value was calculated by considering the weight of the collagen product deprived of its water content and multiplied for the percentage of collagen present in the collagen product in the form of dry matter. The porosity of the collagen products was estimated as the density of the dry collagen per unit volume. This value may be calculated from the weight of the medical device subtracted of the water content, with the saturation occurring under vacuum beforehand. The value so obtained must then be multiplied for the percentage of collagen in the medical device, considered as a dry matter, and finally the resulting value must be divided for the volume of the medical device.

Morphological analysis was undertaken to verify whether hydration may have some bearing on the resolution of collagen fibers in freeze-dried collagen products. Biochemical analysis and the effect of the collagenases of types I and III standard collagen along with samples of Colactive, Biopad, Promogram, and Puracol were resolved by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Electrophoresis was carried out by using a Mini-PROTEAN apparatus (Bio-Rad, Hercules, California) equipped with 2 polyacrylamide gels, with results photographed. Fibroblasts and HLs were selected and allowed to interact with collagen products for up to 48 or 72 hours. Cell culturing of the fibroblast 3T3 cell line was originally obtained by disaggregation of a mouse embryo. Upon becoming fully confluent, cell cultures were split using 0.25% trypsin/EDTA and seeded again at 2 to 5 × 10,000 cells/cm on sterile collagen foils prepared from Colactive, Biopad, Promogram, and Puracol and allowed to grow at 37° C in a 5% CO_2 atmosphere. The cultured cells were tested against the collagen products and removed after 24, 48, and 72 hours to be examined by light and electron microscopy. Lymphocytes (white blood cells) were also obtained from the Blood Transfusion Centre of the Santa Chiara Hospital in Pisa, Italy.

Figure 1.

THICK EPOXY RESIN SECTIONS OF 4 DIFFERENT COLLAGEN SAMPLES EXAMINED AT THE LIGHT MICROSCOPE LEVEL



Sample names are indicated on the pictures themselves. A 50-μm scale is indicated for each micrograph. Condress represents Biopad.

These cell cultures were tested against collagen products removed after 24, 48, and 72 hours to be examined by light and electron microscopy. Analysis of a collagen sample by light microscopy suffers from a major drawback; that is, what in origin belongs to a volumetric 3-dimensional extension is ultimately quantified on a bidimensional level. The structural characteristics that are envisioned under these conditions may not reliably reflect any real difference intervening between samples being tested. To overcome these difficulties, the collagen samples were analyzed by scanning electron microscopy.

RESULTS

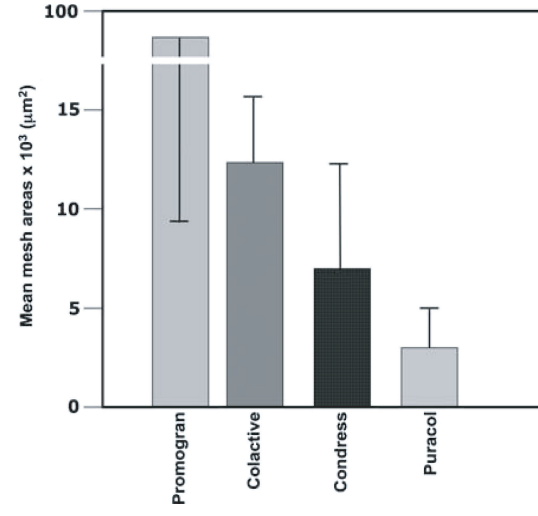
The dimensional characteristics, as well as the dry collagen content of the collagen products examined, are summarized in Table 1. Biopad had the largest collagen content of 100% and porosity of 217% adding to a larger-sized product in comparison with Colactive, Puracol, and Promogran. The overall surface is very similar for all collagen products. When the nitrogen content was correlated with that of the dry matter, the actual value of collagen content could be determined. The known values of the actual nitrogen and hydroxyproline content for each collagen product, together with their humidity content, allowed the authors to estimate the real content of dry collagen in each collagen product. It is interesting to note that the Puracol collagen content determined under this method was 88.4%, which differs from the manufacturer's statement of 100% collagen content. In review of how much collagen is in contact with the wound surface, the highest value for this parameter was Biopad—amounting to about 140% that of Colactive, 400% that of Puracol, and 322% that of Promogran.

The porosity of the collagen products is summarized in Table 1. In regard to porosity, Puracol and Colactive were very low in this parameter, whereas Biopad and Promogran were much higher. However, it is likely that this high value for Promogran might be related to an abnormal ratio between the low quantity of collagen (76.18 mg) present and the volume of this collagen product (7.07 cm^3).

Under light microscope analysis, different collagen samples exhibited varying structural characteristics in regard to the extent of interstitial areas and mesh properties (Figure 1). Promogran and Puracol exhibited the highest and lowest strand extension, respectively, among all collagen samples examined in this study. Biopad and Colactive have intermediate values; however, the former also has thinner collagen strands as supports of its mesh structure. To provide a quantitative analysis of this visual impression, areas enclosed by the collagen strands were calculated for each sample through an image analyzer, and the data obtained this way were reported on the histogram as

Figure 2.

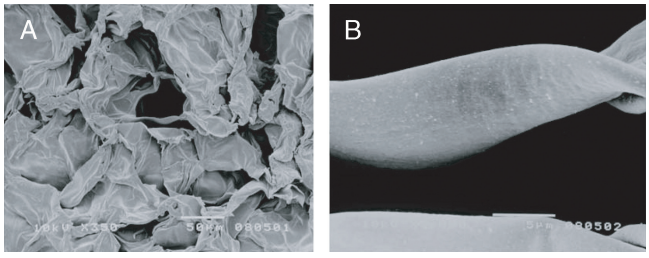
HISTOGRAM SHOWING THE EXTENT OF MESH AREAS ENCLOSED BY COLLAGEN STRANDS FOR EACH OF THE 4 SAMPLES AS DETERMINED THROUGH AN IMAGE ANALYZER ON THICK EPOXY SECTIONS



Condress represents Biopad.

Figure 3A, B.

SCANNING ELECTRON MICROGRAPHS OF COLACTIVE COLLAGEN AS SEEN AT LOW MAGNIFICATION (×350 IN A) AND AT HIGHER MAGNIFICATION (×15,000 IN B)



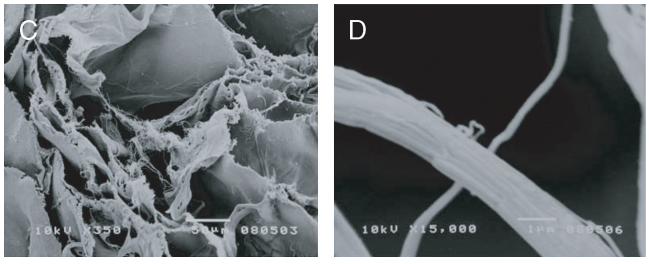
Scale bars are indicated in the micrographs as 50 and 5 μm, respectively.

seen in Figure 2. Promogran had too high of a value for the mesh area, thus indicating that there were too few collagen strands per unit of volume in this sample. The opposite condition was exhibited by Puracol, which has the lowest mesh area due to thinner collagen strands, but more densely distributed within the same area, once again indicating a lower percentage of collagen dry matter per unit of volume. Biopad and Colactive are both within more reasonable values for the mesh areas; however, this value was associated with thicker collagen strands only in Biopad.

Analysis of Colactive by scanning electron microscopy shows that what appear as collagen strands by light microscopy in reality are very thin intermingled laminas (Figure 3A). The extent by which interstices are meshed together is very high, but the collagen strands that delimit this interlaced structure are very thin, amounting to less than 2 μm in thickness (Figure 3B). Figure 3C is a low-magnification picture of the Biopad sample.

Figure 3C, D.

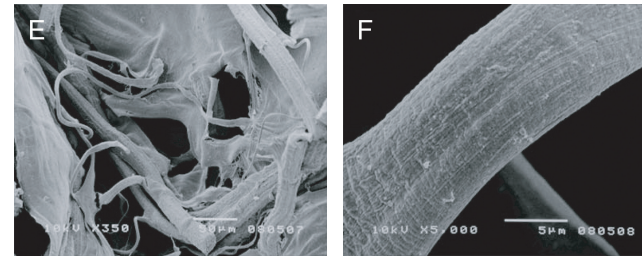
SCANNING ELECTRON MICROGRAPHS OF BIOPAD COLLAGEN AS SEEN AT LOW MAGNIFICATION (×350 IN C) AND AT HIGHER MAGNIFICATION (×15,000 IN D)



Scale bars are indicated in the micrographs as 50 and 5 μm, respectively.

Figure 3E, F.

SCANNING ELECTRON MICROGRAPHS OF PROMOGRAN COLLAGEN AS SEEN AT LOW MAGNIFICATION (×350 IN E) AND AT HIGHER MAGNIFICATION (×5000 IN F)

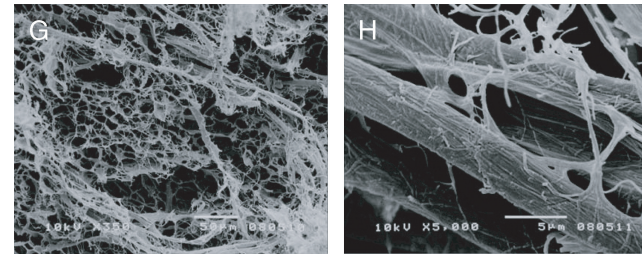


Scale bars are indicated in the micrographs as 50 and 5 μm, respectively. Note that a complex substructure can be envisioned only in the collagen fiber depicted at a higher magnification.

The 3-dimensional extensions of the collagen strands, in this sample, show that laminas are more complex in structure than could be envisioned at first sight by simple light microscope observations. This demonstrates that collagen laminas in Biopad samples are formed by several collagen fibers tightly and densely packed together. Figure 3D shows the detail, at high magnification, of one of these fibers as it leans out from an underneath lamina. At this enlargement, it can be seen that even this fiber has an underlining structure made up of numerous fibrils. Figure 3E is a low-magnification micrograph of the Promogran sample showing several collagen laminas and fibers dispersed within the mesh substructure. What this image shows is that very few laminas are fractured crosswise, indicating and thus confirming that there are too few collagen strands per unit of volume. When examined at a higher magnification, the collagen fibers in this sample exhibit a molecularly complex substructure associated with the thinner fibrils (Figure 3F).

Figure 3G, H.

SCANNING ELECTRON MICROGRAPHS OF PURACOL COLLAGEN AS SEEN AT LOW MAGNIFICATION (×350 IN G) AND AT HIGHER MAGNIFICATION (×5000 IN H)



Scale bars are indicated in the micrographs as 50 and 5 μm, respectively. This is the only collagen sample in which the mesh infrastructure is evidently thicker.

When Puracol was examined with the scanning electron microscope, the structural characteristic that could be appreciated was due to a high collagen density (Figure 3G). For example, a comparison of all the pictures of Figure 3A-H showed that only Puracol has an abnormally higher collagen density. Because this structural condition is also associated with thinner collagen strands, a higher mesh density does not necessarily correspond to higher collagen contents. On the contrary, the opposite condition is more likely to occur. At higher magnification, Puracol shows that collagen laminas are so fragile that they are reduced to thin collagen fibrils cross-linking wider collagen bundles of fibers (Figure 3H).

To verify morphologically whether all samples are truly collagen products, they were processed for transmission electron microscopy for higher-resolution observations. Under these experimental conditions, it is possible to verify whether collagen fibrils are properly assembled and, above all, if they exhibit a clear staggering pattern of black and white bands. A low-magnification micrograph of Colactive showed several collagen strands enclosing some collagen areas. From this evaluation, one can obtain information concerning the actual thickness of the collagen strand that is less than 2 μm. At higher magnification, the collagen strand does not reveal the presence of any banding pattern, thus indicating that either collagen fibrils are not present or, alternatively, that they have not been properly assembled during the extraction procedure. On comparing the electron microscopic evaluation of the Biopad and Colactive products, the collagen strands in the Biopad sample were much thicker, attaining up to 4 μm in thickness at certain joints exhibiting a well-defined substructure that was totally absent in the Colactive sample. At higher magnification, the collagen strand substructure is clearly due to the alternating pattern of black and white bands. Taken together, these observations suggest that collagen fibrils are not only present in due amounts to confer a proper thickness to the collagen strands, but they are also spatially arranged to provide a densely packed collagen. These parameters may have a key role to play in relation to cell invasiveness of the collagen matrix and in relation to the stability of the collagen products. When Promogran samples were examined with the transmission electron, the morphological characteristic that strikes the observer's eye is the actual thickness of the collagen strands. Unlike those of Colactive and Biopad that range to a maximum width of 2 and 4 μm, respectively, Promogran was found to range in strand width from a minimum of 8 μm to a maximum of 12 μm. This is a significant observation, especially if logically linked with the fact that Promogran also has the largest collagen area of all collagen samples examined in this study. These strands show typical collagen fibers with an alternate pattern of black and white bands. Such observations suggest that most of the collagen fibers in the Promogran are densely packed collagen strands. However, because of this dense packaging, the collagen area comprised between adjacent strands is too bound to be too extended. Therefore, this may affect the way this product interacts with invading cells.

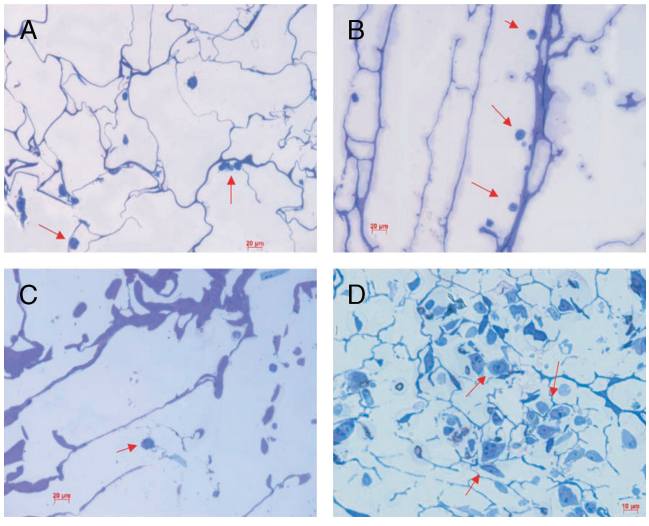
Finally, when Puracol was examined by electron microscopy, both low- and high-magnification pictures revealed the presence of well-staggered collagen fibers. It was also observed that in low-magnification pictures, collagen is primarily associated with fiber bundles of about 4 or 5 μm in thickness. Other very thin fibrils could be seen branching out from these bundles and perhaps delimit the real collagen area. At higher magnification, this collagen sample exhibits very nicely banded fibrils with a mean diameter of about 200 nm each. These observations confirm the authors' first impressions at the scanning electron microscope, that Puracol has a highly dense collagen bordered by thin collagen strands. The microscopic analysis did show that, besides these thin collagen strands, there are also thick bundles of collagen fibers that are not involved in constructing the collagen substructure.

There is a difference on how the 4 collagen products are modified following exposure to collagenase for 5 hours at 37° C. As compared with the control, exposure to collagenase causes both Puracol and Promogran to become apparently deprived of the thinner threads present in the interlaced collagen matrix, thus retaining only some structurally denser spots. Following exposure to collagenase, Promogram appeared to retain only large clumps of a densely packed material. When observed by high-resolution microscopy, both revealed no periodicity regardless of the ultimate instrumental magnification attained. Nonetheless, Promogran collagen exhibits typical periodical fibrils; thus, it is likely that structurally organized fibrils are easily removed by the collagenase treatment and that only clumps of packed fibrils are retained. In contrast, Biopad retains the same overall structural characteristics, even though collagen threads are clearly frayed. Finally, the Colactive samples seem to totally collapse into compact clumps.

To find out more about the structural modifications collagen samples undergo following exposure to collagenase, all collagen samples subjected to collagenase exposure were also examined by electron microscopy. The Puracol collagen product remained almost unaltered even after 5 hours of exposure to collagenase, suggesting that tight fiber packaging may render it almost inaccessible to the enzyme. Unlike the Puracol samples, Biopad was found to retain its overall structural characteristics as they are visible at low magnification. Yet when frayed collagen threads were examined at a higher resolution, they appeared fully disaggregated and much more dispersed than in control samples.

Figure 4.

LIGHT MICROSCOPE SECTIONS OF COLLAGEN SAMPLES (A, COLACTIVE; B, BIOPAD; C, PROMOGRAN; D, PURACOL) EXAMINED AFTER 48 HOURS OF IN VITRO CULTURE IN THE PRESENCE OF 3T3 FIBROBLASTS



Scale of 20 μm is indicated in each picture A, B, C. Scale bar in D is 10 μm.

Interestingly, single collagen fibers are still very well resolved, suggesting that the primary effect of collagenase may be envisioned in the process of collagen desegregation, rather than in fibril disassembly. As already observed by light microscopy, Colactive collagen samples collapsed into large and densely packed clumps whenever exposed to collagenase. When these clumps were examined by electron microscopy, they appeared as large aggregates of randomly dispersed fluffy material. Therefore, a major effect of collagenase exposure with Colactive samples can be envisioned in the aggregation and collapse of all collagen fibrils, rather than in any alteration of the assembly or periodicity of the single fibrils themselves.

To summarize the main observations obtained by electron microscopy on samples treated with collagenase, the following points can be highlighted.

Puracol became deprived of thinner threads of the interlaced collagen matrix, but the high-density fibrils remained almost unaltered; their packaging may make them inaccessible to collagenase.

Biopad retained the same morphological characteristics, but the overall structural appearance of the collagen matrix was much more relaxed. Single collagen fibers, although highly frayed, maintained their typical banding of periodical black and white bands.

Colactive samples were totally collapsed into compact clumps resolvable as large aggregates of randomly dispersed fluffy material by electron microscopy.

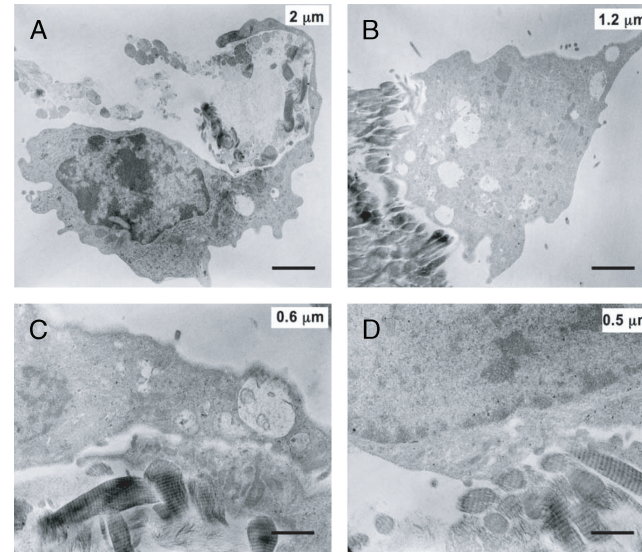
Promogran retained only some structurally dense spots that, unlike those in control samples, are devoid of any structural periodicity.

Given the evidence provided on the structural modification of collagen products as induced by collagenase treatment, and considering the actual role these samples would have to play in wound healing, the authors decided to examine how the products interact with various cell types. The following figures are meant to illustrate how fibroblasts and lymphocytes do indeed interact with the 4 collagen samples that are being compared in this study.

As it can be seen in Figure 4, the overall appearance of the collagen matrix does not change very much in the presence of fibroblasts as compared with the controls. To better understand how fibroblasts interact with the collagen products, each of the 4 collagen products was examined with the transmission electron microscope. Fibroblasts were cocultured in the presence of the Colactive. At low magnification, fibroblasts took firm contact with the surface of collagen fibers. No alteration can be envisioned in the collagen substructure, nor

Figure 5.

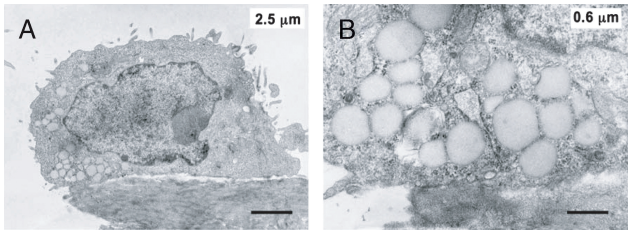
ELECTRON MICROSCOPE MICROGRAPHS OF BIOPAD COLLAGEN SAMPLES EXPOSED FOR UP TO 72 HOURS AT 37° C TO 3T3 FIBROBLASTS IN DULBECCO MODIFIED CULTURE MEDIUM



Scale bars are 2 μm (A), 1 μm (B), 0.6 μm (C), and 0.5 μm (D).

Figure 6.

ELECTRON MICROSCOPE MICROGRAPHS OF PROMOGRAN COLLAGEN SAMPLES EXPOSED FOR UP TO 72 HOURS AT 37° C TO 3T3 FIBROBLASTS IN DULBECCO MODIFIED CULTURE MEDIUM



Scale bars are 2.5 μm (A) and 0.6 μm (B).

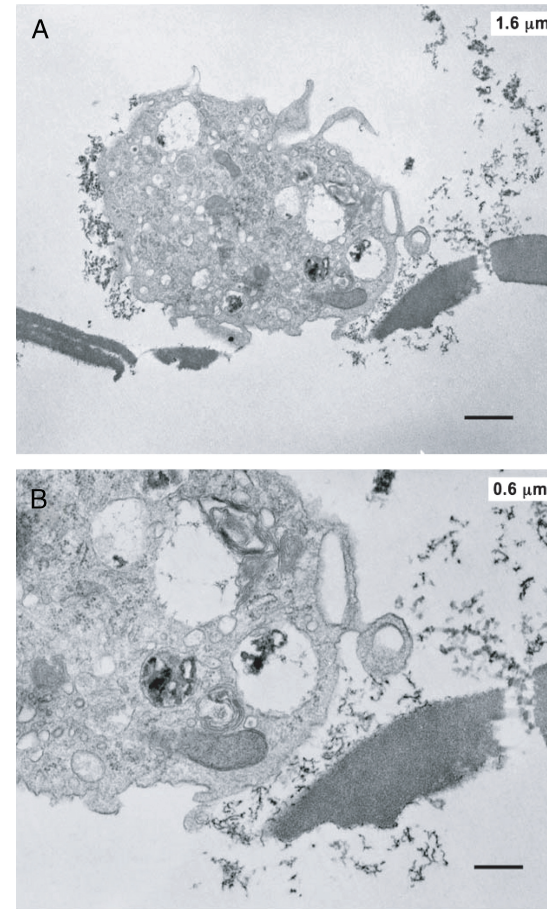
can pseudopodia be seen associated with the fibroblast cell periphery. At a higher magnification, the fibroblast cell membrane shows little contact with the collagen surface and a number of uncoated vesicles that could be either exocytic or endocytic in nature. These observations may indicate that fibroblasts do not interact very easily, at least within the period tested in this study with the Colactive gel, due perhaps to its superficial properties that may make it difficult for the cells to penetrate deeply. 3T3 fibroblasts were placed with Biopad in a coculture for up to 72 hours (Figure 5). Embracement between 3T3 fibroblasts and Biopad collagen may become so tight that several cells may simultaneously be involved in enclosing a cluster of several collagen fibers (Figure 5A). At a higher magnification, one can see how the interaction between collagen fibrils and 3T3 cells is actually attained. Figure 5D shows that this is realized through a direct contact of the cell membrane with a few collagen fibrils that become consequently frayed. Occasionally, collagen fibrils can be seen so close to the cell surface as to give the impression of being internalized by endocytosis. Figure 5C demonstrates that some electron-dense material has gained access to the cytoplasm of a 3T3 fibroblast cell. In addition, the presence of several multivesicular bodies in the proximity of the cortical cytoplasm strengthens the impression that something is being modified intracellularly. Whether this is functionally equivalent to a collagen proteolytic process taking place cannot be clearly said in the absence of proper markers. The observations of Biopad demonstrated that fibroblasts have an active behavior on this collagen product and may contribute to remodeling by either helping to degrade or to synthesize some collagen fibrils.

When the Promogran collagen sample was examined ultrastructurally in the presence of 3T3 fibroblasts, results were similar to those already depicted for the Colactive sample (Figure 6). Although 3T3 fibroblasts take firm contact with the Promogran collagen surface, they never form pseudopodia for locomotion, nor do they form any membrane infoldings or protrusions indicative of an active interaction. Thus, even in this case, the Promogran sample appears so densely packed that the fibroblasts are somehow prevented from entering the gel matrix and establishing a more elaborate participation in either degrading the collagen or synthesizing new collagen fibrils.

Figure 7 shows a sequence of micrographs documenting Puracol interaction with fibroblasts. It can be observed that collagen fibers are more or less unaltered, and in a few points, they take contact with the fibroblast surface (Figures 7A and B). When these contact points are examined at a higher magnification, no special feature of the cell plasma membrane can

Figure 7.

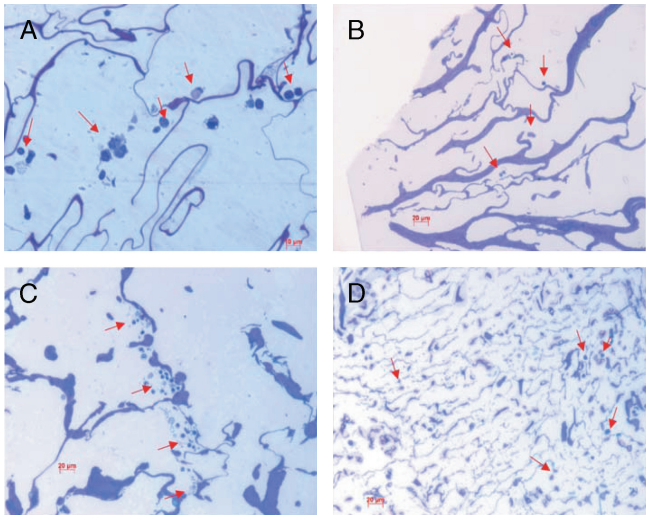
ELECTRON MICROSCOPE MICROGRAPHS OF PURACOL COLLAGEN SAMPLES EXPOSED FOR 72 HOURS AT 37°C TO 3T3 FIBROBLASTS



Scale bars are 1.6 μm (A) and 0.6 μm (B).

Figure 8.

LIGHT MICROSCOPE MICROGRAPHS OF SEVERAL COLLAGEN SAMPLES: (A), COLACTIVE; (B), BIOPAD; (C), PROMOGRAN, AND (D) PURACOL CULTURED IN VITRO FOR UP TO 72 HOURS IN THE PRESENCE OF HUMAN LYMPHOCYTES



Red arrows point to the sites where lymphocytes are located. Bar scales are 20 μm for all pictures.

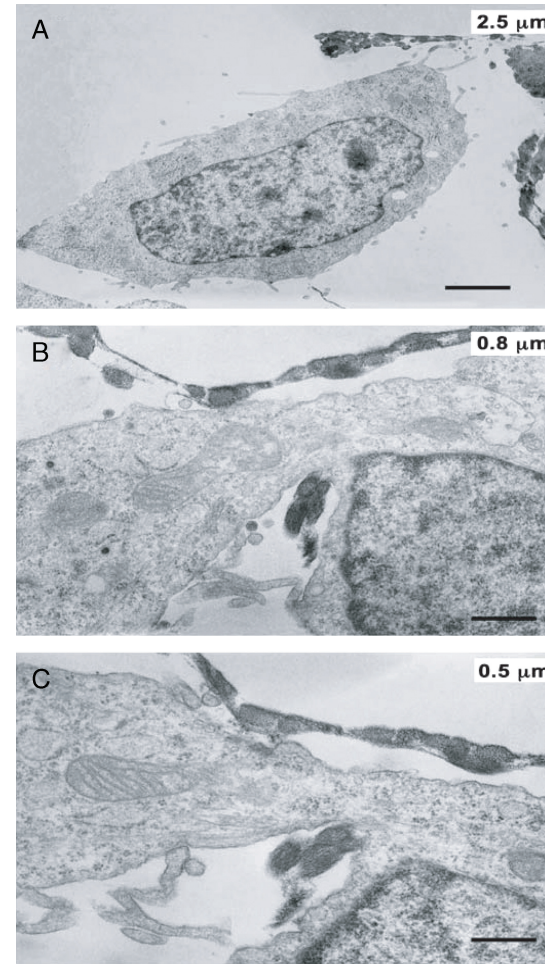
be envisioned. Once again, even this sample of Puracol appears to react rather passively with fibroblasts, for no sign of any alteration either in the cell or in the collagen itself can actually be found.

To further ascertain the potential role played by these collagen products in wound healing, the authors tested their ability to interact with human white blood cells, mainly lymphocytes. Figure 8 depicts a series of 4 light microscope micrographs documenting this interaction. At this low magnification, no collagen alteration can be envisioned. To verify whether blood cells or the collagen matrix itself may actually be structurally modified as a result of this interaction, all samples were examined with a higher resolution at the electron microscope. As it can be clearly seen in Figure 9, the Colactive product can actually undergo several structural modifications when exposed to lymphocytes. In particular, it is noted that the periphery of the large clumps of collagen fibrils is disaggregated in the proximity of the lymphocyte surface (Figure 9A). In addition, the lymphocyte itself is highly modified intracellularly because it exhibits a number of endosomal vesicles, some of which include materials structurally similar to the disaggregated collagen fibrils (Figure 9B). The authors take this observation as an indication that lymphocytes participate actively in the process of wound healing by partially degrading and most likely internalizing all collagen fibrils with which they may come into contact.

When the Biopad collagen sample was examined by electron microscopy, an even more pronounced pictured emerged, in that lymphocytes appeared more heavily loaded with electron-dense material in their endosomal compartment. In addition, the collagen fibers present in the extracellular milieu appeared highly reduced into clumps of fibrillar material. On the whole, these observations demonstrate that the Biopad collagen is highly susceptible of being degraded following interaction and internalization by lymphocytes.

Figure 9.

ELECTRON MICROSCOPE MICROGRAPHS OF COLACTIVE COLLAGEN SAMPLES EXPOSED FOR 72 HOURS AT 37° C TO HUMAN LYMPHOCYTES



Scale bars are 2.5 mm (A), 0.8 mm (B), and 0.5 mm (C).

This conclusion is based primarily on several structural findings, but they would certainly require more sophisticated techniques to identify the nature of the material being produced and internalized as a result of the collagen-lymphocyte interaction. When this ultrastructural study was extended to the analysis of the Promogran collagen sample, the authors could observe that the collagen and the interacting lymphocytes were not at all altered morphologically or only slightly modified in their superficial appearance. Although a few plasma membrane indentations can actually be envisioned along the lymphocyte surface, no structural modification can be seen to occur in the gel matrix, nor is any endosomic vesicle seen to appear in the lymphocyte cytoplasm.

Puracol was examined by electron microscopy following interaction with lymphocytes with no modification in the collagen matrix, nor could any change be seen to occur in the lymphocyte cytoplasm. The authors interpreted the previous observations as indicating that Promogran and Puracol are much more resilient to lymphocytes in that they persist longer unaltered whenever they come into contact with the lymphocytes.

CONCLUSION

Table 1 summarizes the composition, shape, percent collagen content, surface area, and porosity of Biopad, Colactive, Puracol, and Promogran. The parameters to be considered are the actual dimension of the collagen substructure and the thickness of the collagen strands. Collagen products too large or too small may not be suitable for sustaining any cell movement during the healing process. At the same time, collagen strands that may be either too thin or too thick may compromise the actual persistence of the medical device on the wounded area, because of low collagen content per unit volume. The evidence provided in this study demonstrates how Biopad, Colactive, Puracol, and Promogran can actually interact differently with the enzymes and the cells that characterize the environment of a healing wound. The application of several morphological and biochemical techniques with the 4 different collagen products allowed us to draw the following conclusions. All samples are characterized by an interlaced matrix bordered by collagen strands.

In the authors' opinion, the Biopad collagen sample exhibited the best structural compromise between the extension of the collagen areas and the thickness of the collagen strands because this collagen product demonstrated to be the only collagen product evaluated that was capable of retaining the same overall structure during exposure to collagenase and at the same time to allow the collagen interlaced matrix to be clearly frayed off. This may be one of the best conditions for sustaining specific interactions for wound healing, as the product will maintain its structural integrity for a longer period, allowing longer interaction of the collagen with the wound. This should allow a greater likelihood of healing the wound. The scanning electron microscope demonstrated that the Biopad collagen strands could be seen as made of thick laminas of packed fibers, whereas all other samples had either strands or fiber bundles that are too highly and densely packed. When examined at a higher resolution, all collagen products, except Colactive, revealed the presence of a staggered pattern of black and white bands. In addition, at this level of analysis, the authors could see how the collagen fibrils were packed together to make up the collagen strands of the interlaced matrix. Too many fibrils make the collagen strands too thick, as in the case of Promogran. In contrast, fewer fibrils make the collagen strands too thin and fragile to persist during the healing process. Collagen fibers too loosely packed may accelerate collagen product degradation. Conversely, collagen fibers packed too densely may not allow significant wound fluid absorption and also not allow significant interaction of the entire collagen product with the wound, having a negative impact in healing.

In regard to collagen matrix modification, Colactive and Biopad appear to be sufficiently well altered when placed in contact with lymphocytes, suggesting that they may be easily accessible during the wound healing process and rapidly degraded. Of these 2 samples, the Biopad collagen sample appears to be involved in a more active interaction with the fibroblasts, as signified by the appearance of several membrane indentations along their cell contour. The other 2 samples differ both in their capacity to degrade collagen and to sustain any active interaction with the fibroblasts. ●

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