SOCIETY FOR BIOMATERIALS
2019 ANNUAL MEETING & EXPOSITION
APRIL 3-6, 2019 • WASHINGTON STATE CONVENTION CENTER • SEATTLE, WA

Mis-concepts about Collagen in Regenerative Biomaterial Matrices

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Introduction:
Collagen as a biomaterial has its potential in wounds & burns management, ophthalmic, orthopedics, and tissue engineering applications. Moreover, collagen plays a major role in the delivery of therapeutic proteins, drugs, genes and stem cells for tissue repair and regeneration. There seems to prevail certain mis-concepts about this exciting biological protein as listed below: Structural configuration, surface modifications like crosslinking, inclusion of contaminants of other immunogenic molecules like elastin and immunogenic collagen types etc. For example, products made of denatured collagen (like gelatin) and the broken peptides of collagen are all allowed to be described as collagen products without realizing the biological impact of such molecules in terms of their bioactivity towards tissue regeneration and remodeling. Also the intact tissue derived products that are made from the membranes of amnion, pericardium, intestinal wall, urinary bladder etc. They all must be contaminated with approximately 15% Elastin, type-III collagen, lipids and other proteo-glycans which are all highly immunogenic. All such products have to be cross-linked to minimize their immunogenicity. While doing so, biologically valuable type-I collagen contained in those products are also chemically modified and loses the bioactivity and other binding abilities that significantly impairs the tissue regenerative and wound healing abilities.
The purpose of this study is to compare the commercially available "Helicoll collagen membrane" (claimed to be made of high purity native type-I collagen) with other biological skin substitute categories that are generally referred as "other collagen products". Helicoll product is the result of the unique enzymatic process patented by Encoll Corporation, CA, USA, yielding a highly purified collagen that is relatively non-immunogenic. The collagen is further bioactivated by moderate amounts of phosphorylation, a post-translational modification of collaged.
Majority of the proteins involved in cell activation are subjected to reversible phosphorylation. Tyrosine phosphorylation has been proven to activate the cells during cell signal transduction.
The significance of protein phosphorylation is to induce cell signal transduction through a cascade of enzymatic reactions. It is a well known fact that the collagen which is the largest native structural natural protein present at the sites of tissue repair or remodeling or growth. Phosphorylation of collagen makes the molecule biologically more active and becomes essential for the cell signal transduction to happen. Collagen has specific binding regions for all active components like cell membrane receptors, ligand, platelets, growth factors and other cytokines for proper interaction tha t would result in repair, remodeling and regeneration of tissues. Phosphorylaed collagen plays an important role due to its ability to bring all necessary factors together and to activate them for the desiredl result. Additionally, the phosphorylated collagen tends to attract the divalent cations like Ca and Mg. Such divalent cations are essential for activating the platelets and other physiological events (Ref.) for faster wound repair or tissue growth.

Materials and Methods:
Experimental assays have been developed using cultured tissue derived from rabbit corneal epithelium to study migration of epithelial sheets during wound closure and cell-substrate adhesion. Corneal epithelial cell cultured layer punch model[2] with certain modification has been used for this study. Morphology of the injured area was analyzed by measuring main parameters of the wound shape, including area, perimeter and diameter. Punch injuries followed by epithelial debridement using a disposable plastic tip (i.e., 1.0, 1.5 and 2.0 mm punch) produced consistent shapes and values. Results are displayed as mean + /− SD (N = 9). Significance was determined using one-way ANOVA with Bonferroni's post-hoc test. *p < 0.05; ***p < 0.001. ns, non-significant.
Another model[3] to study wound closure, epithelial defects, 6 mm in diameter, were produced in vitro in 24 well multiplates by a local freezing technique, and the size of the remaining defect was quantitated over time by staining. Thus, cultured corneal epithelial cells used to assay for influences on the migratory events governing closure of superficial epithelial wounds.

Results Conclusions & Summary:
Test articles include the following: Helicoll™, Fish Collagen Prep., Cadaver Skin Prep., Porcine Intestinal Mucosa, XL Collagen Dressings, Amnion based matrix. The results showed significant advantage to the Helicoll over other test articles used.
Due to space restrictions the detailed results are not shown here. However the following table depicts how Helicoll Collagen may differ from other Collagen Products:

FEATURE	*Helicoll™*	Fish Collagen Prep.	Cadaver Skin Prep.	Porcine Intestinal Mucosa	XL Collagen Dressings	Amnion based matrix
Pure Type-I	Yes	No	No	No	No	No
Nativity of Collagen	Yes	No	No	No	No	No
Healing Rate	High	Low	Low	Low	Low	Low
Native Attachment Sites	High	Low or None	Low or None	Low or None	Low or None	Low or None
Potential to Buffer Excess Glycosylation	High	Low or None	Low or None	Low or None	Low or None	Low or None
Potential Cell Signaling	High	Low or None	Low or None	Low or None	Low or None	Low or None

References:
1. Gunasekaran, S. Collagen-Based Wound Dressings: How to Control Infection and Immune Response. In Donald Wise et al, ENCYCLOPEDIC HANDBOOK OF BIOMATERIALS AND BIOENGINEERING, Marcel Dekker Inc., NY, 1995, 2(A), 1267-1284.
2. Gonzalez-Andrades, M. et al. (2016) Establishment of a novel in vitro model of stratified epithelial wound healing with barrier function. Nature / Sci. Rep. 6, 19395; doi: 10.1038/srep19395 (2016).
3. Jumblatt, MM, Neufeld AH A tissue culture assay of corneal epithelial wound closure Investigative Ophthalmology & Visual Science 02/1986; 27(1):8-13