

Update

A Resource for Veterinary Surgeons

Products and Ideas
for Intramedullary
and External
Skeletal Fixation

Hall Griffin, DVM
Jeff Kellerman, DVM
Craig Woods, DVM

Advanced Veterinary BioScaffold

Regenerative medicine is a rapidly emerging field in human and veterinary surgery. IMEX™ has partnered with TR BioSurgical, LLC to make innovative,

two decades of clinical practice, research and development experience to this issue. Dr. Woods has conducted basic and applied research in cartilage repair, orthopedics,

KNOWN LIMITATIONS BY CLASS

Synthetic Bone Substitutes
(calcium phosphates, bioglass)



- Many are radiopaque
- Poorly osteoinductive
- Variable resorption (months)

Demineralized Bone Products
(cadaver sources)



- Potentially immunogenic

Bone Morphogenic Proteins



- Expensive
- Supraphysiologic amounts are required

Collagens



- Not osteoinductive
- Requires bone morphogenic proteins to augment performance

Chart 1a

regenerative technologies for tissue repair affordable and available to the veterinarian. One such technology is a new veterinary medical device, called TR Matrix™. The matrix, or bioscaffold, structurally resembles embryonic extracellular matrix, but contains no embryo derived tissue. It is composed of naturally occurring substances. This pivotal structural difference differentiates TR Matrix™ from all other tissue repair products currently available to veterinarians.

Craig Woods, DVM and Jeff Kellerman, DVM of TR BioSurgical, LLC are guest coauthors of this newsletter and bring over

osteoarthritis, and has helped develop a number of biomaterials for veterinary medicine. Dr. Kellerman is a seasoned clinician and is responsible for the TR Matrix™ clinical trials.

REGENERATIVE MEDICINE

Regenerative medicine products are generally categorized into cell-based technologies (stem and progenitor cells) or bioscaffolds which mimic extracellular tissue and may provoke a regenerative tissue response. Although cell based approaches show considerable promise, they remain expensive and often require

IN THIS ISSUE

- 16th Complete Course in External Skeletal Fixation
- Advanced Veterinary BioScaffold



TRMatrix™
Lyophilized (1 gram)



processing of the cells, which can prevent immediate treatment. This has prompted scientists to develop bioscaffolds that permit cells to integrate and form new tissues. Although bioscaffolds can be used in a variety of tissue types, this newsletter will focus on use in the context of orthopedics.

Currently, most orthopedic bioscaffolds consist of calcium phosphates, demineralized bone materials, and collagens alone or in combination with growth factors (platelet derived growth factors, morphogenic proteins). Unfortunately, all of these bioscaffolds have limitations briefly described in Chart 1a (see cover page).

THE SOLUTION

These limitations are overcome with TR Matrix™, whose properties conform to the ideal bone repair scaffold. TR Matrix™ regenerates multiple connective tissues. It is osteopromotive, non-immunogenic, easy to administer, radiolucent, cost-effective, has a short resorption period, a long shelf life, and is sterile. When cells interact with this bioscaffold they upregulate growth factors in a physiological manner.

OSTEOGENESIS

TR Matrix™ bioscaffold is indicated for repair of fractures, bone defects, delayed unions, osteotomies and non-unions in canine, feline, and equine patients. TR Matrix™ may also be used as a cancellous bone graft extender. As shown in figure 1 (right), TR Matrix™ provokes osteogenesis in a critical size ulnar defect model.

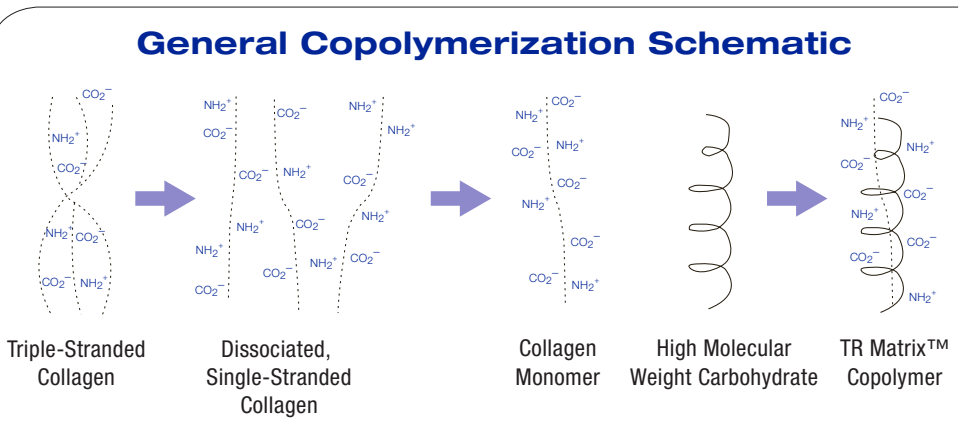
Based on the osteogenic effects observed in critical size defect models, TR BioSurgical is now engaged in several canine and equine orthopedic clinical studies. TR BioSurgical is currently studying its effects in tibial plateau leveling osteotomy (TPLO) and fracture repair.

THE STRUCTURE OF TR MATRIX™

The simple structure of TR Matrix™ is

responsible for its properties. Standard extracellular matrix components are reverse engineered to make TR Matrix™ resemble tertiary embryonic connective tissue^{9, 10}, which is open and not folded. This important and unique feature is not found in other bioscaffolds. This open configuration exposes molecular sequences, termed matrikines, which are important for tissue regeneration.

linked extracellular matrix and less differentiated cells in the wound bed; reduced wound contraction; rapid and sustained neovascularization; and scarless wound healing. TR Matrix™ has been shown to alter the type of extracellular matrix produced in the healed wound bed from a highly cross-linked, dense structure normally found in healed adult wounds to a more open reticular structure found in healed fetal wounds. As a result,

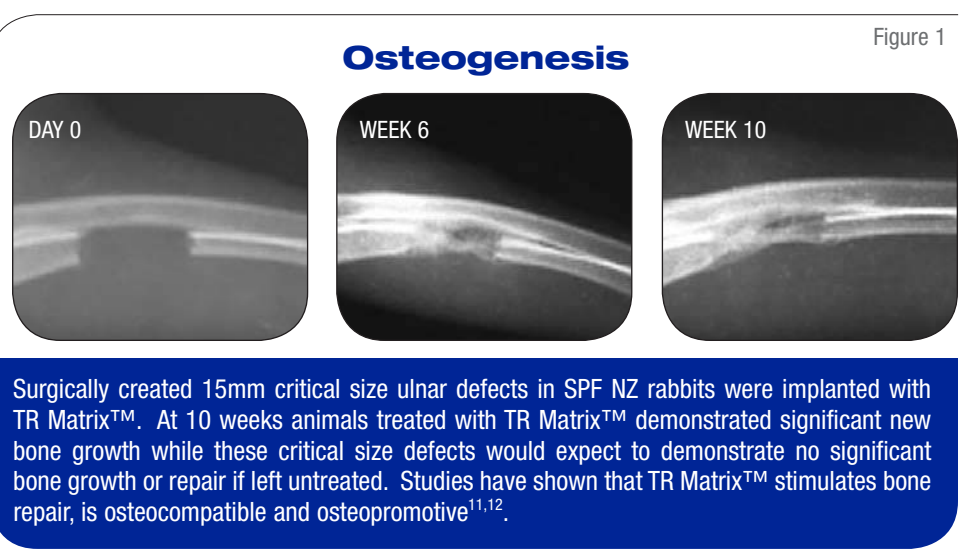


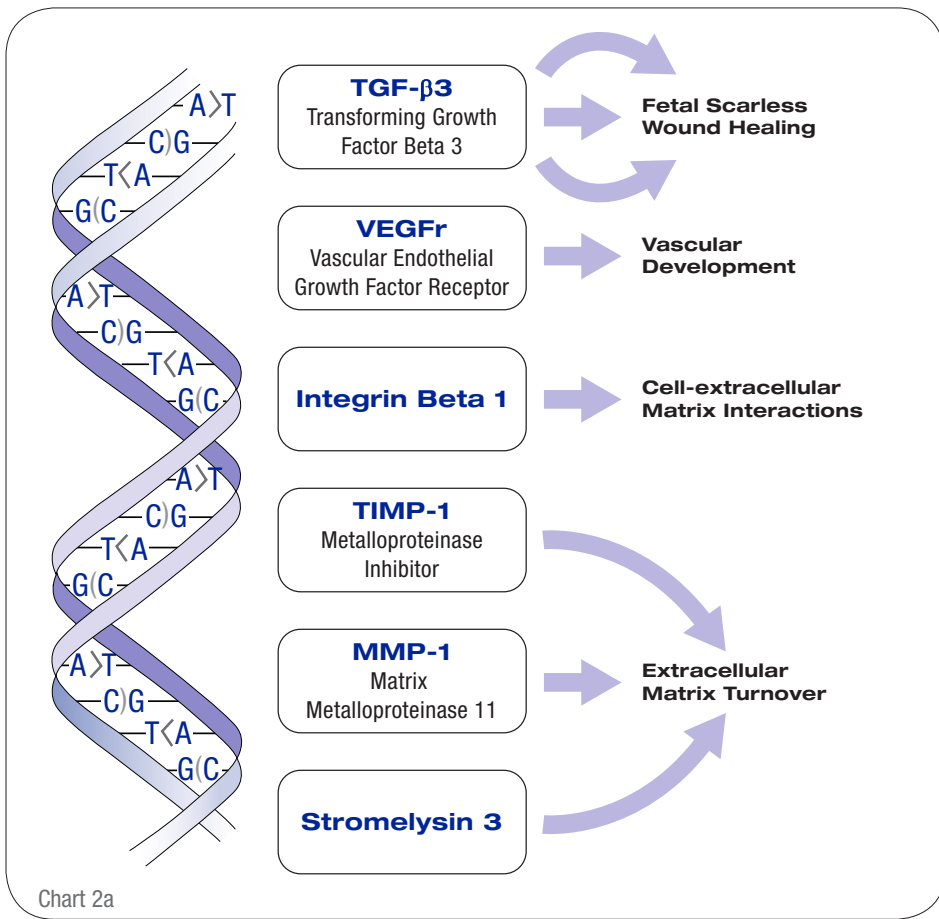
Triple-stranded collagen is untwisted to an open polar configuration, a configuration that resembles tertiary embryonic connective tissue. This open polar configuration is retained by copolymerizing the denatured collagen with a polysaccharide.

FETAL-LIKE REPAIR MECHANISMS

Fetal wound healing is characterized by an altered pattern of response compared to adult wound healing. Adult healing is dependent not on tissue regeneration, but on wound contraction which results in significant scar tissue formation. In contrast, the hallmarks of fetal wound healing include: a less tightly cross-

there is less wound contraction and fibrosis. In addition, TR Matrix™ initiates a sustained vascular response (angiogenesis^{1, 2, 3}) in contrast to the transient increase in vascularization seen with growth factors^{1, 2, 3}. It is noteworthy that TR Matrix™ results in significantly reduced scarring during tissue repair^{5, 6}, a process termed fetal-like repair⁴.





vasculogenesis^{6, 7, 8, 13}. In vitro gene regulation studies have demonstrated this effect^{6, 7, 8, 12}.

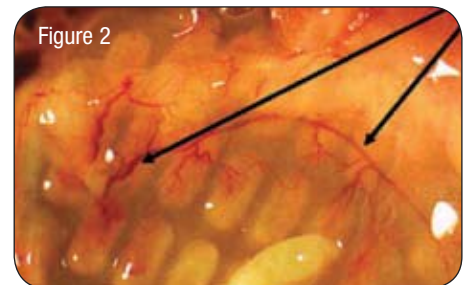
TR Matrix™ has the ability to upregulate a variety of genes involved in tissue repair, as evidenced by gene microarray analysis (Chart 2a). TR Matrix™ also has significant, measurable increases in select repair factors at 3 to 5 hours after treatment with TR Matrix™ compared to control, as measured in standard cell culture assays (Chart 2b).

Interestingly, upregulation of TGF- β 3, an embryonic growth factor, is thought to result in scarless regeneration of tissue and a sustained vasculogenesis to enhance tissue regeneration.

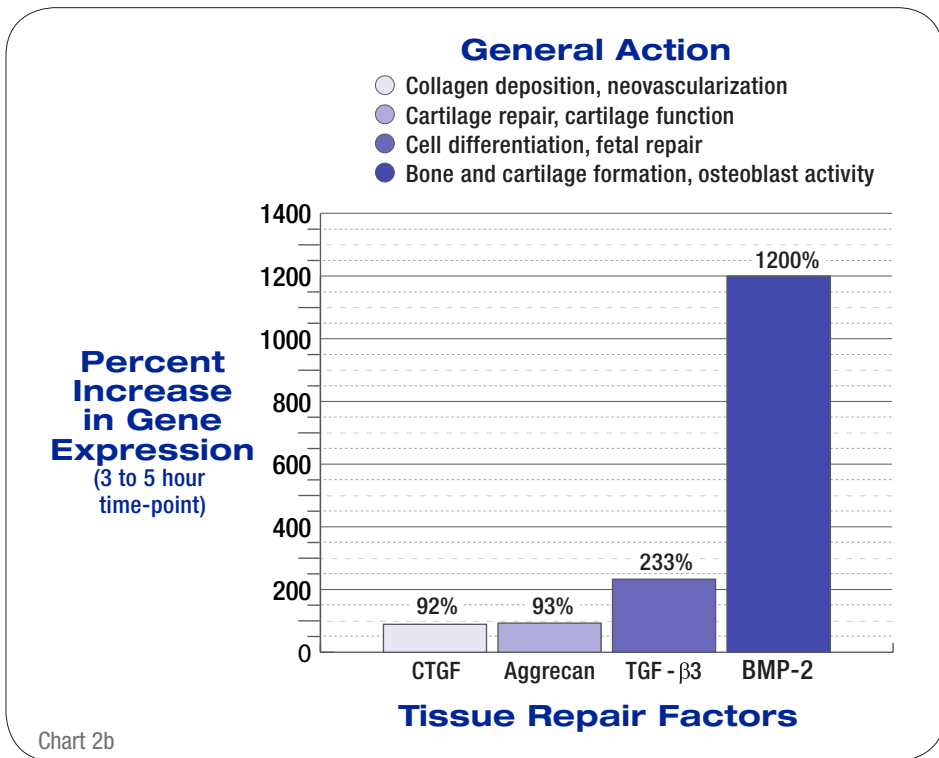
In summary, the innovative, open polar configuration of TR Matrix™ gives it the ability to stimulate physiological repair mechanisms, a very substantial advantage of TR Matrix™ compared to all other bioscaffolds.

VASCULOGENESIS

Vasculogenesis is the term used for new blood vessel formation where no pre-existing vessels were present. Vasculogenesis is an indicator of endothelial progenitor cell recruitment to create new blood vessels.



TR Matrix™ was evaluated for its ability to induce vasculogenesis, which is one of the most interesting properties of TR Matrix™. The image above (Figure 2) clearly shows evidence of neovascularization (arrows) around implanted devices, a phenomenon consistent with stem and progenitor cell activity. This is another important quality of TR Matrix™ and is likely a key component of the tissue regeneration observed after implantation.



BIOLOGICAL PROPERTIES OF TR MATRIX™

Depending on the tissue type, cells that bind to the TR Matrix™ bioscaffold increase gene expression

of BMP-2, TGF- β 1 and β 3, connective tissue growth factor, aggrecan and other repair signals. These factors are important for cellular ingrowth, extracellular matrix turnover and

SOFT TISSUE HEALING

Chronic wounds were treated in studies conducted in diabetic dogs as a model for wound repair in human diabetic patients. In controlled studies, diabetic dogs treated with TR Matrix™ had significant reductions in scar formation, supporting a fetal-like tissue repair response^{5,10}.

HISTOLOGICAL APPEARANCE

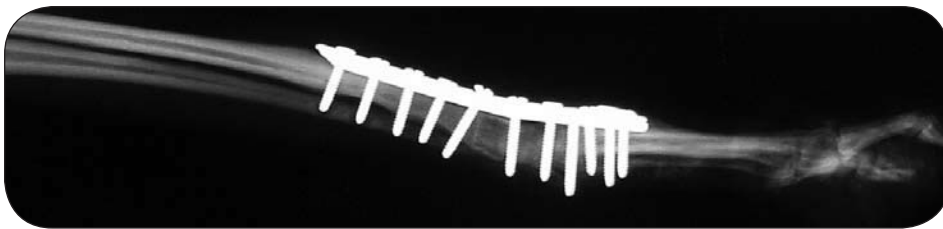
Histologically, surgical incisions also demonstrate improved cellularity and vascular supply after treatment with TR Matrix™. Untreated wounds were dense, fibrous, and avascular. In contrast, TR Matrix™ wounds were well vascularized with less fibrosis and were nearly identical to control skin^{5,6}.

TR MATRIX™ SAFETY

TR Matrix™ has undergone extensive preclinical biocompatibility and safety testing without significant toxicity or safety concerns. The testing included:

1. ISO Acute Systemic Toxicity (mice)
2. ISO Acute Intracutaneous Reactivity (rabbits)
3. ISO Sensitization Study (guinea pig)
4. Cytotoxicity Study (agarose overlay model)
5. Genotoxicity: Bacteria Reverse Mutation Study
6. Genotoxicity: Mouse Bone Marrow Micronucleus Study
7. Genotoxicity: Chromosomal Aberration Study in Mammalian Cells
8. Safety Evaluation of Sub-Q Administration (rat)

Arthrodesis



TR Matrix™ was used to facilitate healing in a canine patient with a failed carpal arthrodesis. The TR Matrix™-treated carpal arthrodesis clearly demonstrates extensive bridging and osteogenesis. (Image courtesy of LeeAnn Blackford, Blackford Veterinary Surgery Referral, Knoxville, TN.)

TR MATRIX™ PRODUCT CONFIGURATION and USES

TR Matrix™ is currently available in packages containing one gram of sterile, lyophilized powder in glass vials. It can be applied as a powder or reconstituted with autologous blood, serum, or sterile saline to achieve the desired consistency for introduction into bony lesions. Prior to reconstitution, TR Matrix™ is stable for up to 1 year and does not require refrigeration or special handling.

REFERENCES

1. Long- and short-term effects of biological hydrogels on capsule microvascular density around implants in rats. Ravin AG, Olbrich KC, Levin LS, Usala A-L and Klitzman B. J Biomed Mater Res. 58(3): 313-8, 2001.
2. Rapid induction of vasculogenesis and wound healing using a novel injectable connective tissue matrix. Usala A-L, Klann R, Bradfield J, Ray S, Hill R, De la Sierra D, Usala M, Metzger M, and Olson G. Diabetes 49 (Suppl. 1): A395, 2000.

3. Long-term neovascularization and capsule thickness surrounding implants coated with hydrogel matrices. Presented at the North Carolina Tissue Engineering Interest Group Meeting, July 7, 1999, North Carolina Biotechnology Center, Research Triangle Park, NC.

4. Induction of fetal-like wound repair mechanisms in vivo with a novel matrix scaffolding. Usala A-L, Dudek R, Lacy S, Olson J, Penland S, Sutton J, Ziats N, and Hill R. Diabetes 50 (Suppl. 2): A488, 2001.

5. Decreased scarring of surgical wounds in vivo following treatment with a novel biopolymer. Lacy SA, Penland SL, Sutton JC, Usala A-L, Olson J, Harris G and Hill RS. The 2nd International Conference and Exposition of Engineering Tissue Growth, March 19-21, 2002 Pittsburgh, PA.

6. A novel hydrogel copolymer reduces scar formation and increases TGF-β3 gene expression. Klann R, Lloyd W, Lacy S, Sutton J, Usala A and Hill R. Platform Presentation at the 15th Annual Symposium on Advanced Wound Care, April 27-30, 2002 Baltimore, MD.

7. DNA microarray analysis of gene expression changes in human skin fibroblasts treated with [TR Matrix™], a novel wound healing hydrogel formulation. Klann RC, Lloyd WH, Sutton JC and Hill RS. Presented at, NIDDK/NIAID/NHLIB Workshop, Advanced Topics in Microarray Analysis, January 22, 2003 Bethesda, MD.

TRMatrix™
Lyophilized (1 gram)

\$149

Part Number
TRM1X1

800-828-4639
www.imexvet.com



8. Selective Induction of TGF Beta 3 as a Marker for Scarless Regenerative Healing in the Skin. Klann RC, Lloyd B, Sutton J and Hill R. Presented at North Carolina Tissue Engineering Interest Group Meeting, June 20, 2003 NC Biotechnology Center, RTP, NC.

9. EMatrix™: A Fetal-Like Extracellular Scaffold for Tissue Integration. Klann RC, Lloyd WH, Sutton JC and Hill RS. Presented at the Gordon Research Conference on Biomaterials and Tissue Engineering, Plymouth, NH, July 20-25, 2003.

10. Fetal-like Tissue Scaffolding as a Substrate for Regenerative Tissue Repair. Klann RC. Invited Speaker,

East Carolina University Brody School of Medicine, Department of Anatomy and Cell Biology Seminar Series. November 12, 2003.

11. A Novel Biomatrix Stimulates Healing in Rabbit Ulna Critical Size Defects. Hill, RS, Enterline, D, and Shih, M-S. Presented at 31st European Symposium on Calcified Tissues, 4-6 June 2004.

12. A Novel Biopolymer Matrix Induces BMP-2 Production and Stimulates Bone Repair in Critical Size Ulnar Defects. Klann, Richard C., Lloyd, William H., Sutton, Jereme C., Shih, Mei-Shu, Enterline, Dave S., and Hill, Ronald S. Platform presentation,

Regenerate 2004, Seattle, WA, June 9-12, 2004.

13. Tissue-specific Response to a Fetal-like Extracellular Matrix: Differential In vitro Gene Expression Associated with Regenerative Wound Repair. William Lloyd, Rich Klann, Jereme Sutton, and Ron Hill. Presented at North Carolina Tissue Engineering Interest Group Meeting, September 30, 2004 NC Biotechnology Center, RTP, NC.