organized by NanoBusiness Commercialization Association, National Nanomanufacturing Network

Nanomanufacturing Summit 2013

& 12th Annual NanoBusiness Conference

Nanobiotechnology for Tissue Engineering and Regenerative Medicine

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Bionanotechnology IP - Based Translational Projects

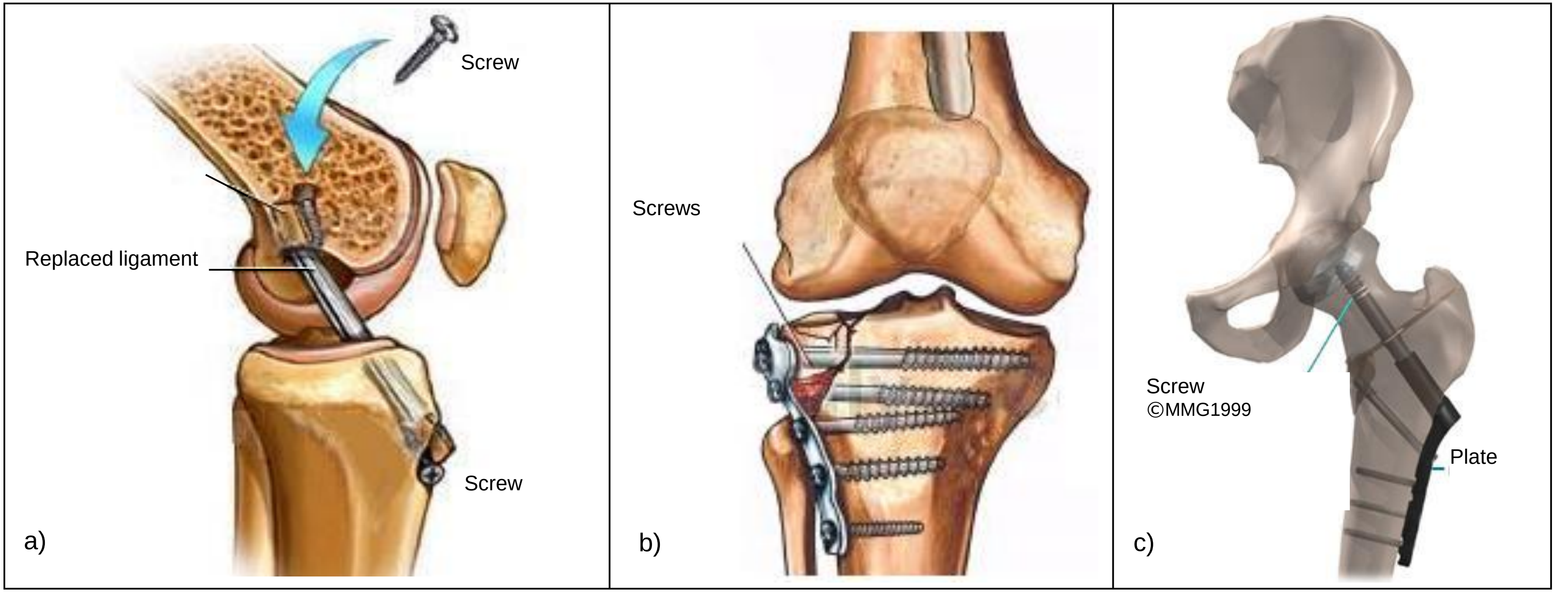
- Nanodiamond-Reinforced Biodegradable Orthopedic Surgical Interference Devices
- Hydroxyapatite Nanoparticles-Containing Electrospun Chitosan Nanofibers for Craniofacial Regenerative Engineering
- LCL-Grafts: Electrospun Biohybrid Small Diameter Vascular Grafts
- Electrospun Soy Protein Scaffolds for Enhanced Wound Healing

Nanobiotechnology for Tissue Engineering and Regenerative Medicine

1. Nanodiamond Reinforced Poly-l-Lactic Acid Scaffolds for Orthopedic Surgical Fixation Devices

2. Electrospun Soy Protein Scaffolds for Enhanced Wound Healing

Surgical Fixation Devices



Metal

- ***Advantage***
- Can provide high initial fixation strength and early rehabilitation in postoperative recovery
- But…

- ***Disadvantage***
- Graft laceration
- Image distortion on MRI
- May require second surgeries for removal

Biodegradable Polymer

- ***Advantage***
- Have proved to be biologically safe to provide adequate graft fixation and
- Overcome the drawbacks of metallic scaffolds
- But…

- ***Disadvantage***
- Breakage during insertion…

K.D. Shelbourne, et. al. American Journal of Sports Medicine, 1990. 18(3), 292-299.
T. Katja, et. al. The Journal of Arthroscopic and Related Surgery, 2006, 22(9), 993-999.
P. Q. Ruhe, et. al. J Biomed Mater Res, 2005, 74A, 533-544.
S. Yang, et. Al. Tissue Engineeingm, 2001, 7(6), 679-689.

Challenge

- The mechanical properties of biodegradable polymers are not strong enough for the requirement of human cortical bone applications.

↓

- A promising strategy to improve the mechanical properties of a polymer is the incorporation of inorganic particles or fibers into the organic matrix of the polymeric structures

W. E..Teo, et al.,. J Biotechnol, 2006, 1, 918-929.

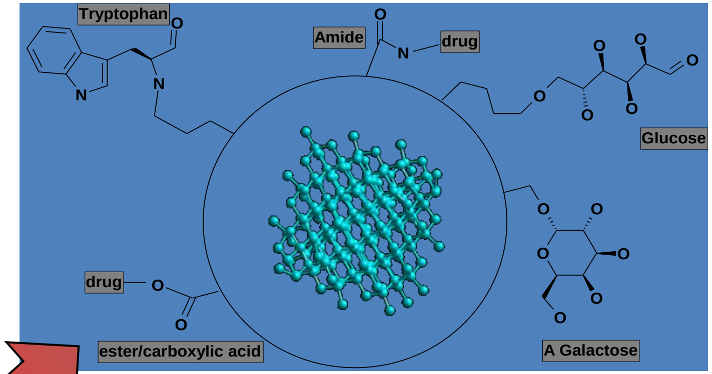
Solution: Reinforcement with Nanodiamonds

Anke Krueger, Chem. Eur. J. 2008, 14, 1382-1390.

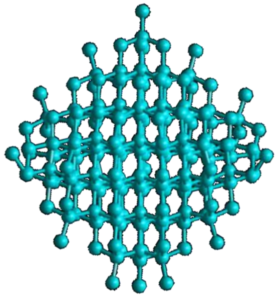
Applications of Nanodiamond

Lubricant additive

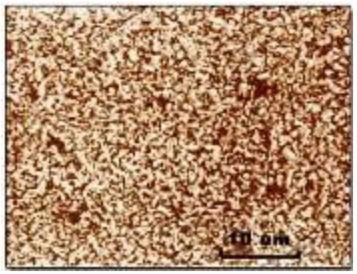
Micropolishing



Biomedical/Pharma

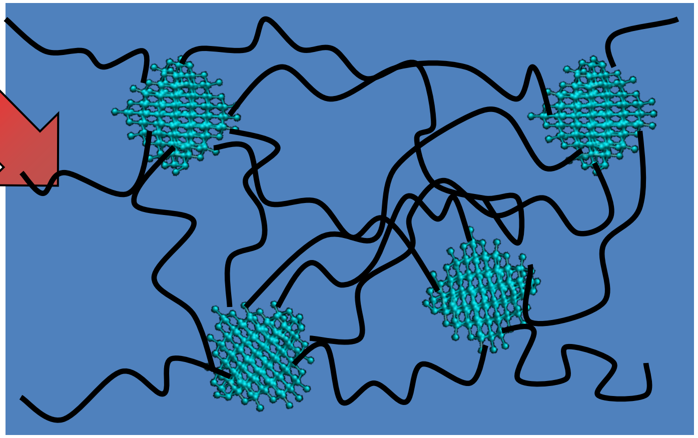


Nanodiamond



Catalyst support

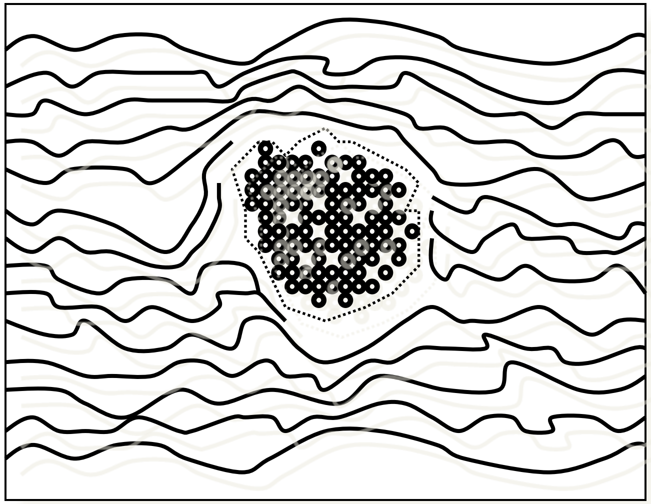
MORE



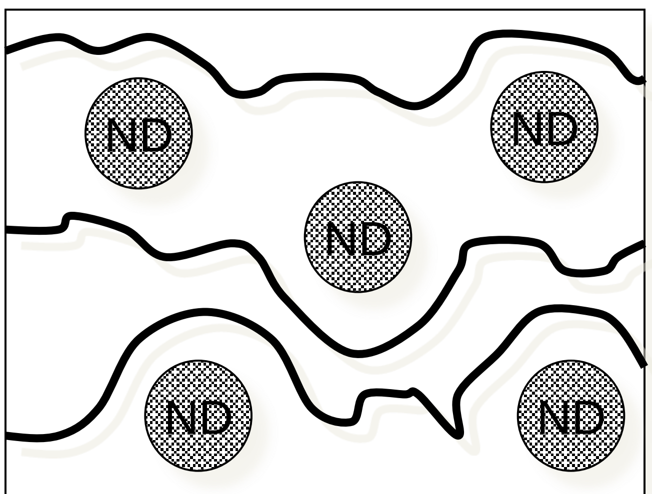
Polymer or metal-matrix composites

V.Y. Dolmatov (2007) Uspekhi Khimii 76(4): 375-397
O. Shenderova, G. McGuire Nanocrystalline Diamond in Nanomaterials Handbook, Yury Gogotsi (ed.), 2006, CRC Press
*H.E.Huang et al. (2007) Nano Lett. **7**(11): 3305-3314*

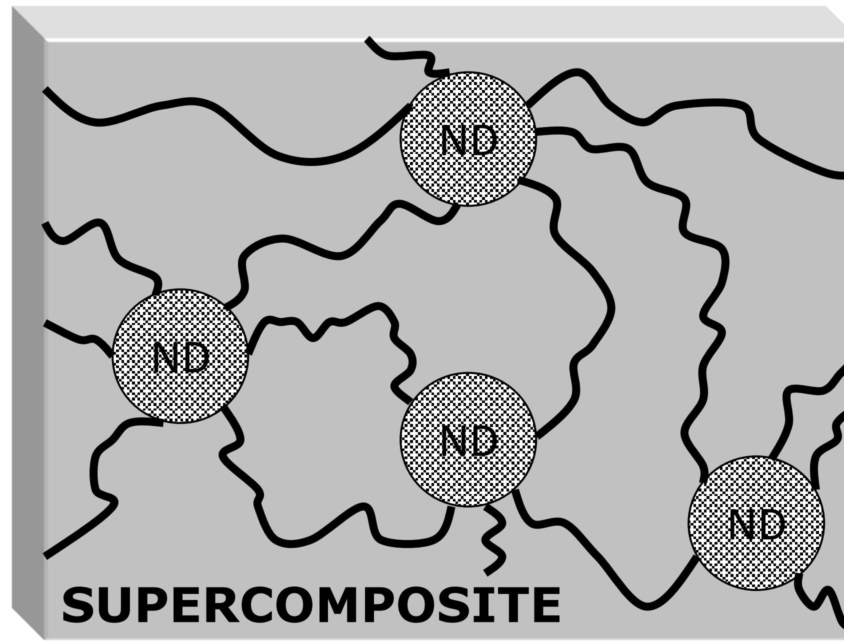
Bulk Nanocomposites: Problems and Solutions



Poor dispersion (agglomeration)

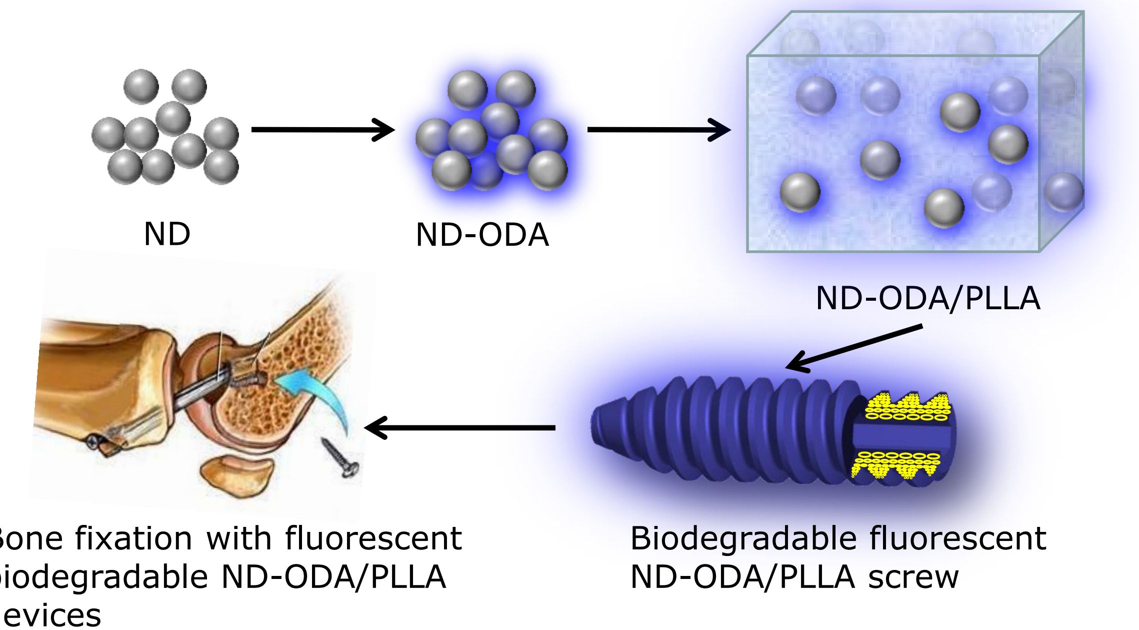


Poor bonding and no load transfer because of weak interfaces



Good dispersion (no agglomerates) and strong covalent bonding between the matrix and the filler

ND-ODA/PLLA nanocomposites for surgical fixation devices: **DESIGN**

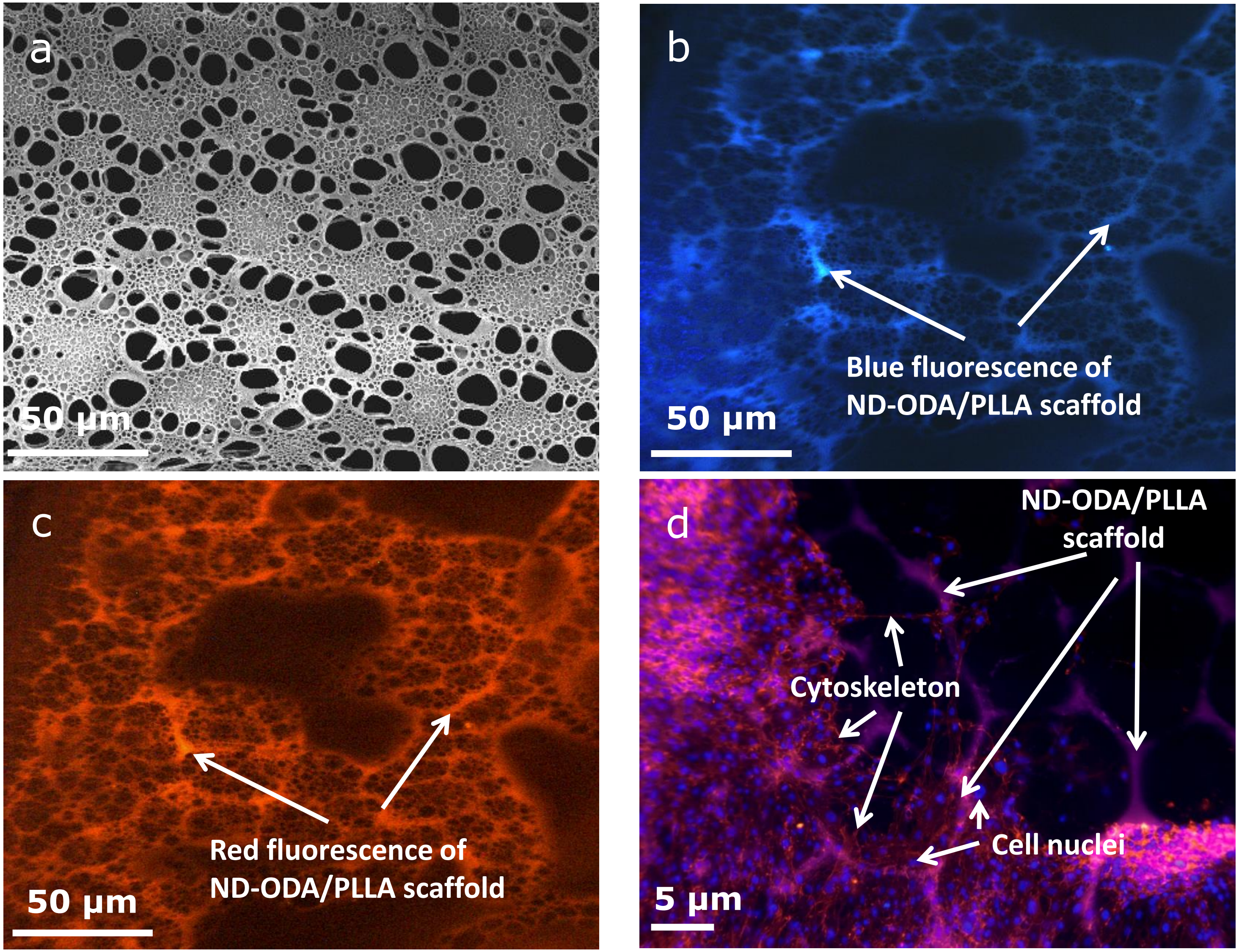


Materials:

✓*PLLA------- poly(L-lactic acid)*

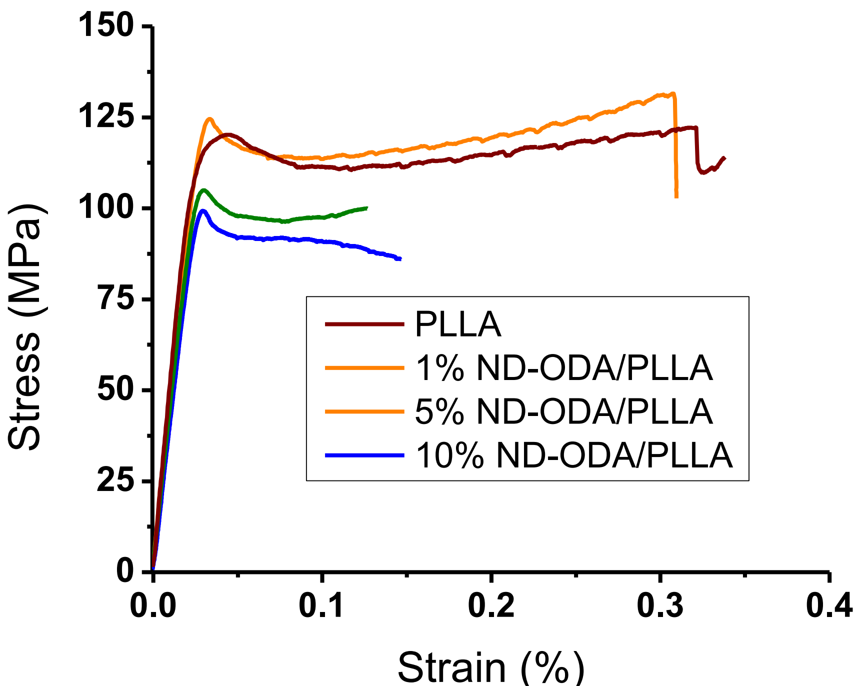
✓*ND-ODA---ocatdecylamine (ODA) functionalized nanodiamond*

ND-ODA/PLLA nanocomposites for surgical fixation devices: fluorescence

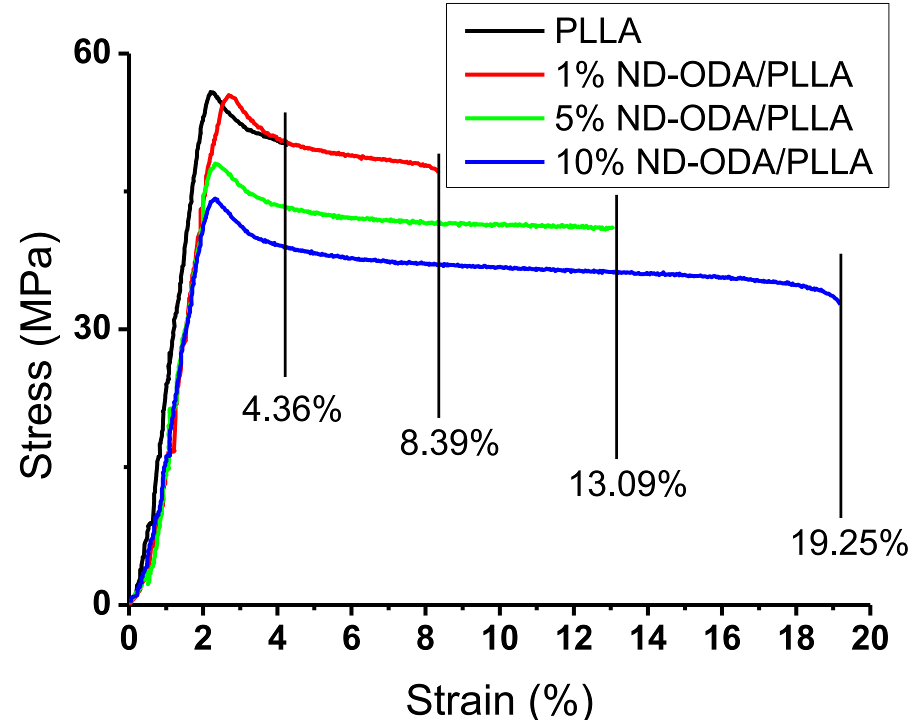


ND-ODA/PLLA nanocomposites for surgical fixation devices: compression and tensile tests

Compressive stress-strain curve



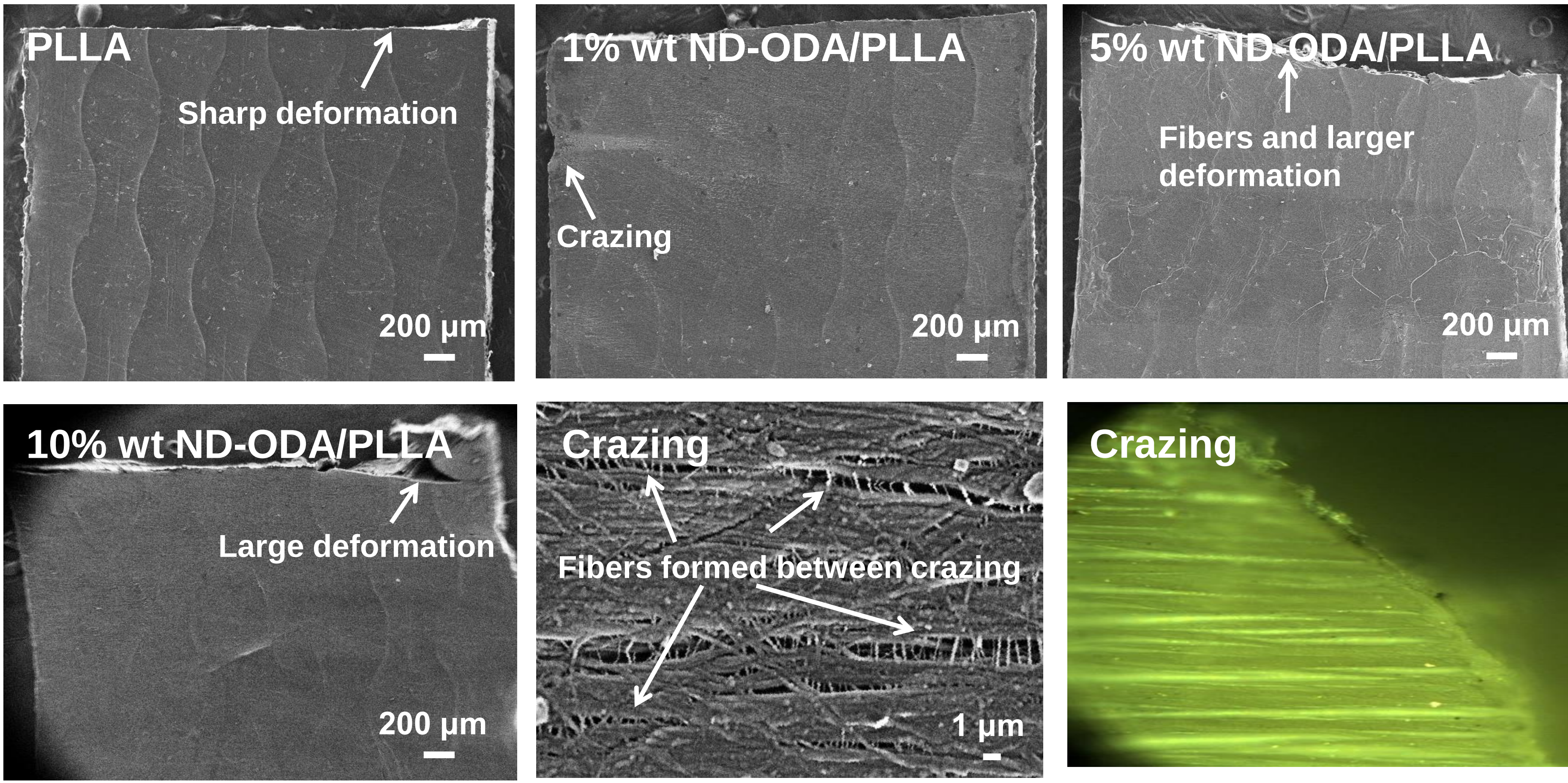
Tensile stress-strain curve



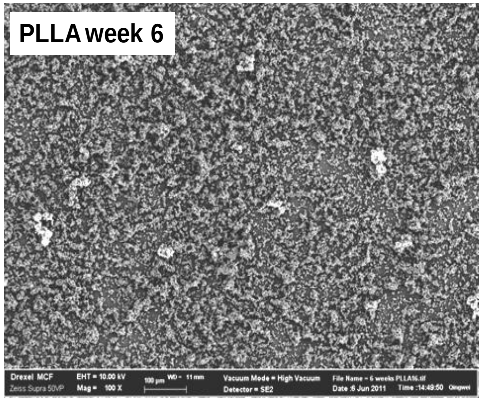
Summarized mechanical properties of the nanocomposites determined from compression test by MTS machine and tensile test by Instron testing machine

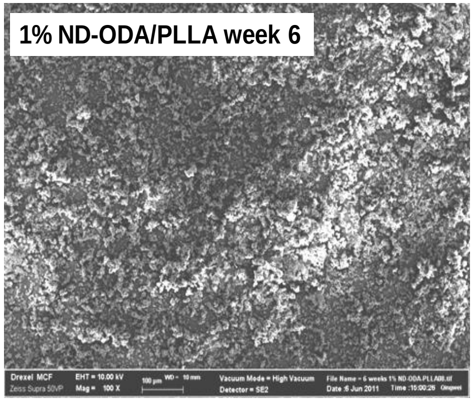
Sample Composition	Young's modulus from compression (GPa)	Compressive strength (MPa)	Relative tensile modulus (GPa)	Ultimate tensile strength (MPa)	Strain at break (%)	Fracture Energy (J)
PLLA	4.38±0.42	118.4±15.2	3.08±0.22	52.85±2.39	4.94±1.14	198.49±64.41
1% wt ND-ODA/PLLA	4.70±0.47	114.4±22.8	2.77±0.22	54.41±1.48	8.29±3.69	637.25±77.38
5% wt ND-ODA/PLLA	4.85±0.45	116.7±8.9	2.89±0.42	48.36±3.18	13.46±4.6 9	721.01±44.24
*- Data are expressed 10% wt ND-ODA/PLLA	5.36±0.49	116.0±15.5	2.69±0.17	45.12±1.68	18.62±1.6 3	941.51±230.7 3

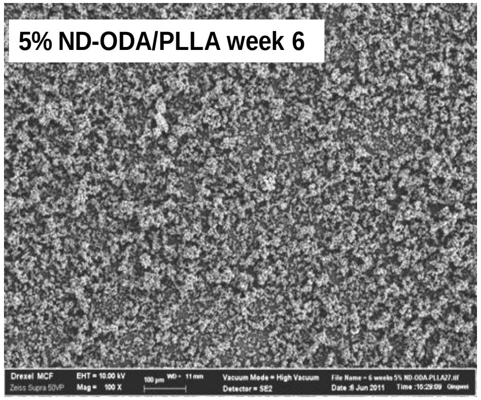
Images of ND-ODA/PLLA nanocomposites after tensile tests

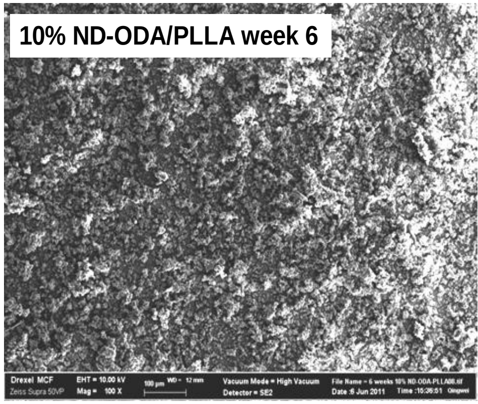


ND-ODA/PLLA nanocomposites for surgical fixation devices: **biomineralization**

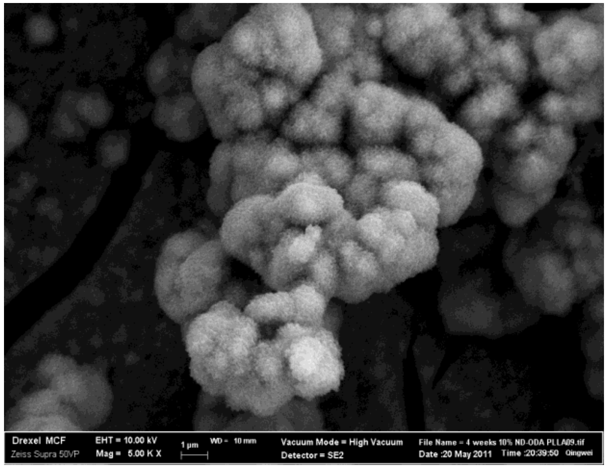


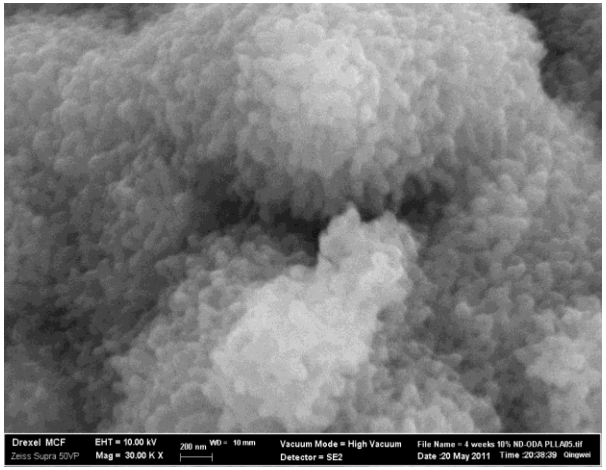


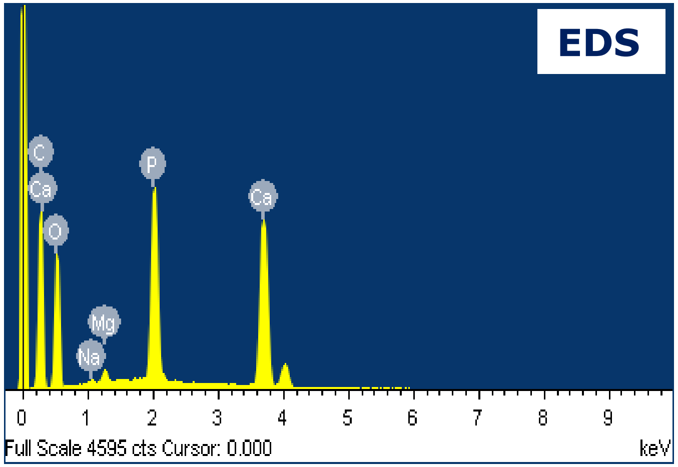




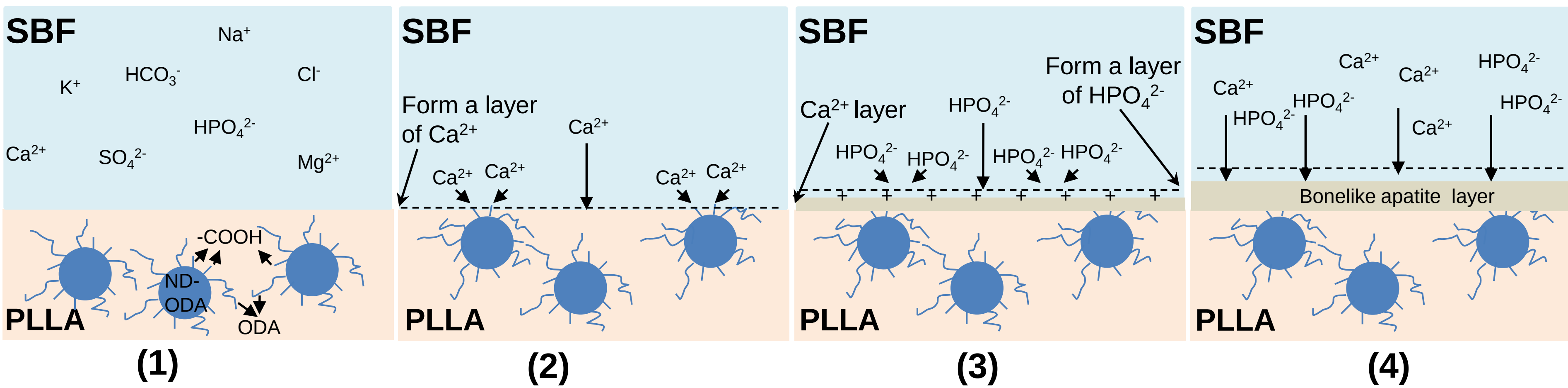
Higher resolution SEM images of mineral cluster formed on 10% wt ND-ODA/PLLA scaffold







A possible mechanism of ND-ODA/PLLA biomineralization



One possible biomineralization process on ND-ODA/PLLA scaffolds in SBF. (1) The initial situation. (2) While contact with SBF, PLLA starts degradation, where -COOH group will form on the surface of the scaffold. With the presence of ND-ODA, the degradation of PLLA may expose the ND-ODA to SBF. The possibly existed -COOH group on ND-ODA will contact with the surrounding SBF. Besides, the ND-ODA may speed up the degradation of PLLA to produce more -COOH group on the PLLA surface. The negatively charged surface will attract calcium ions deposition. (3) The deposited calcium ions will in turn interact with phosphate ions in the SBF and further form bonelike apatite. (4) The bonelike apatite will then grow spontaneously consuming the calcium and phosphate ions to form apatite clusters.

Conclusions

1. ***Enhanced Materials Properties:***
    1. *ODA functionalized ND can achieve uniformly dispersion in PLLA matrix.*
    2. *Hardness of ND-ODA/PLLA composites improves from 320 to 820% upon addition of 1 to 10% wt of ND-ODA.*
    3. *Addition of 10% wt of ND-ODA resulted in more than 380% increase in fracture toughness, which is crucial for manufacturing and use of surgical fixation devices.*

2. ***Enhanced Biocompatibility/Bioactivity***
    1. *The addition of ND-ODA* ***into*** *PLLA matrix does not lead to cytotoxicity and demonstrates biocompatibility*
    2. *The ND-ODA/PLLA scaffolds show enhanced biomineralization*
    3. *Fluorescence for in vivo tracing*

3. ***ODA/PLLA composites are biocompatible and can be used for tissue engineering and regenerative purposes.***

Nanobiotechnology for Tissue Engineering and Regenerative Medicine

1. Nanodiamond Reinforced Poly-l-Lactic Acid Scaffolds for Orthopedic Surgical Fixation Devices

2. Electrospun Soy Protein Scaffolds for Enhanced Wound Healing

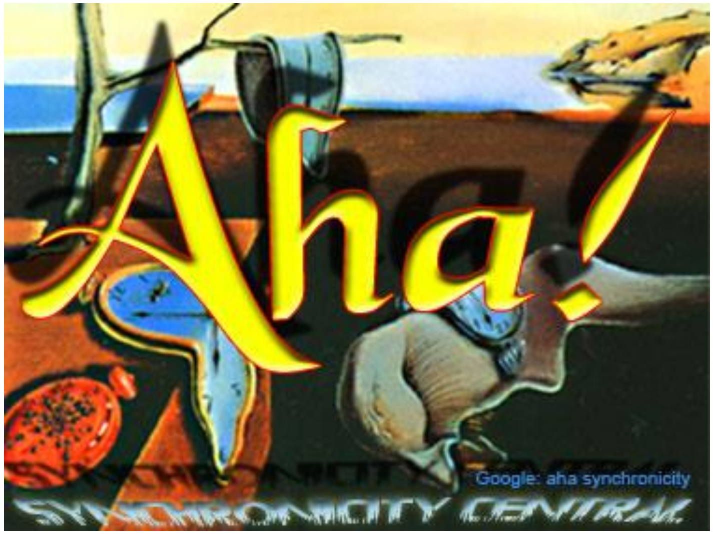
Soy - Soy Milk - Soy Milk Skin

http://www.nandyala.org/mahanandi/?p=501

Fuzhu- aka YUBA - Tofu skin

- also known as dried beancurd, yuba or bean skin is a Chinese and Japanese food product made from soybeans.
- During the boiling of soy milk, a film or skin composed primarily of a soy protein-lipid complex forms on the liquid surface.
- The films are collected and dried into yellowish sheets known as tofu skin or soy milk skin

Fuzhu - Scaffolds!!!!!



Integra Artificial Skin

Clinical Problem: Non-healing Wounds

- Large burn wounds or lacerations (acute)
- Diabetic ulcers (chronic)
- Annual global wound care expenditures amount to $13 – 15 billion[1]

[1] *Fonder et al., J Am Acad Dermatol. 2008,* ***58****:185-206*

Clinical Need I

- There is an unmet clinical need for simple, first-response bioactive wound dressings that would help reduce the morbidity and mortality from severe injury to the skin

- Need for inexpensive, bioactive smart material that promotes wound healing better/faster than current standard of care
  - (good, moist wound care)

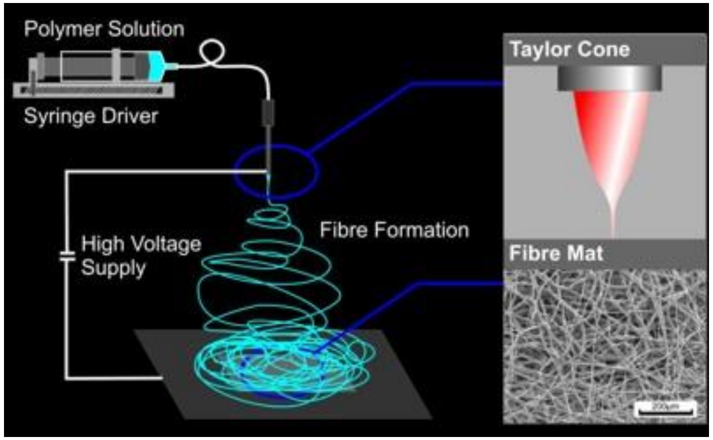
Clinical Need II

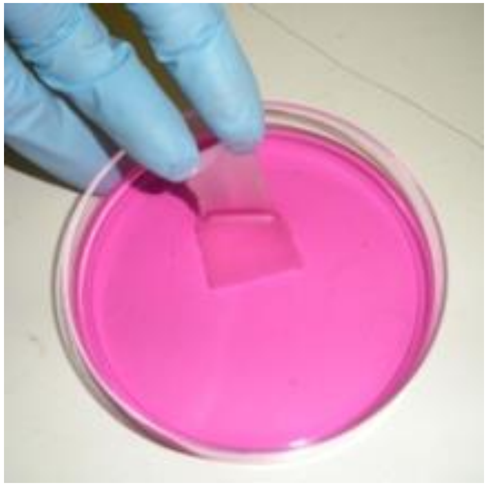
- Acts via “active wound healing” (increase in collagen + angiogenesis)
- Ideally results in scar-free wound healing
- Non-animal derived materials
- Significantly less expensive than current technologies

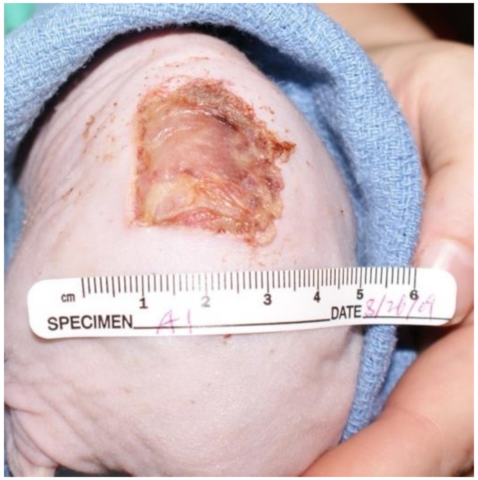
Proposed Solution

- To develop and commercialize “green” plant-derived Alimentary Protein-based Scaffolds (APS, aka OmegaSkin^{TM}) as a readily, available-off-the-shelf acellular scaffold / skin substitute which will promote accelerated wound healing.
- We are testing the hypothesis that soy-bean protein based APS will improve/accelerate wound healing over that provided by the current standard of care using a “regular” full-thickness wound in a rat model with prolonged wound-healing and in pigs.

Bioactive Skin substitutes from Electrospun Soy Protein: OmegaSkin™







Technical Basis

Basic IP / Technology: Electrospinning of Soy Protein

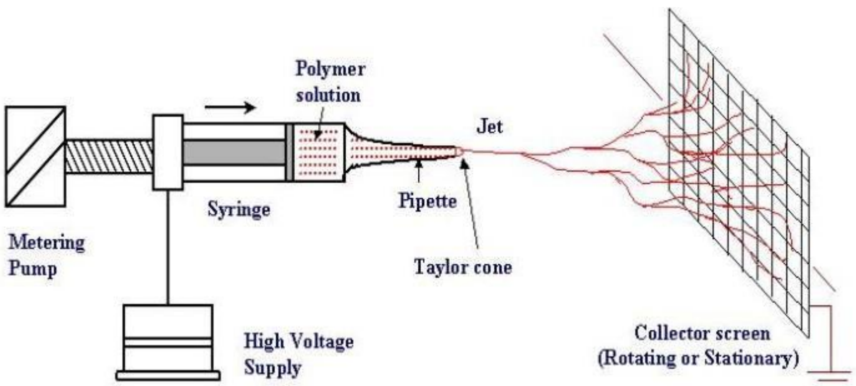
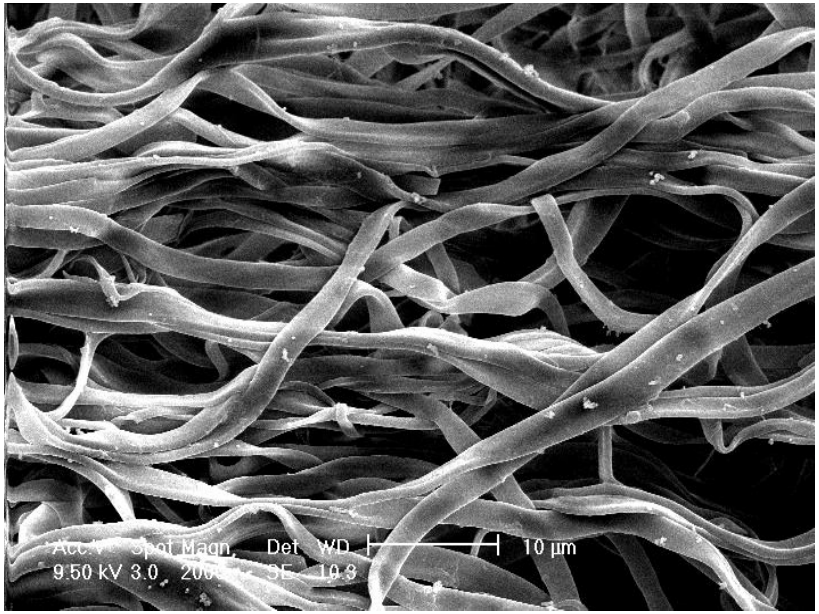
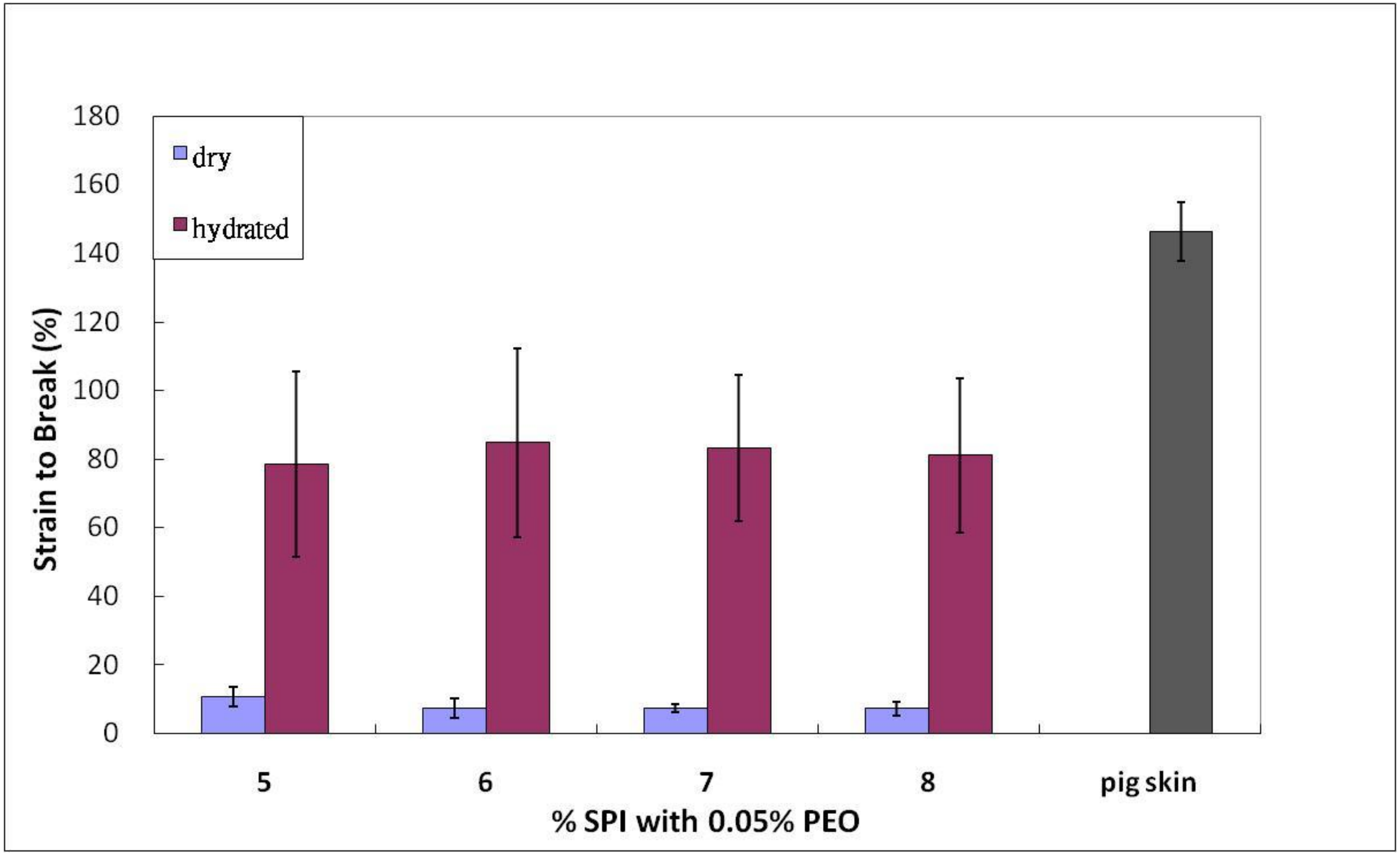


Figure 1. Schematic of the Electrospinning setup.

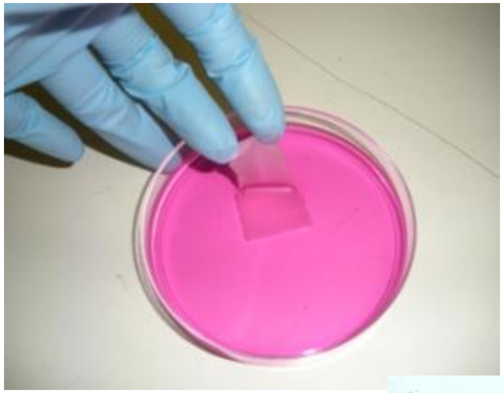


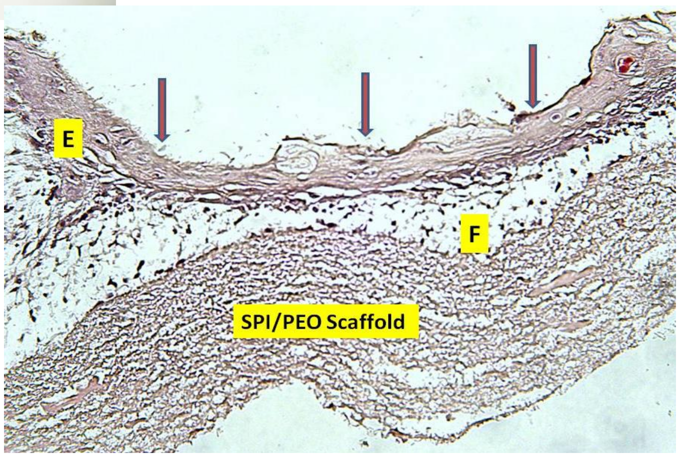
Other Manufacturing Technologies are being considered

Mechanical properties of OmegaSkin™



OmegaSkin™: Skin Substitutes from Electrospun Soy Protein



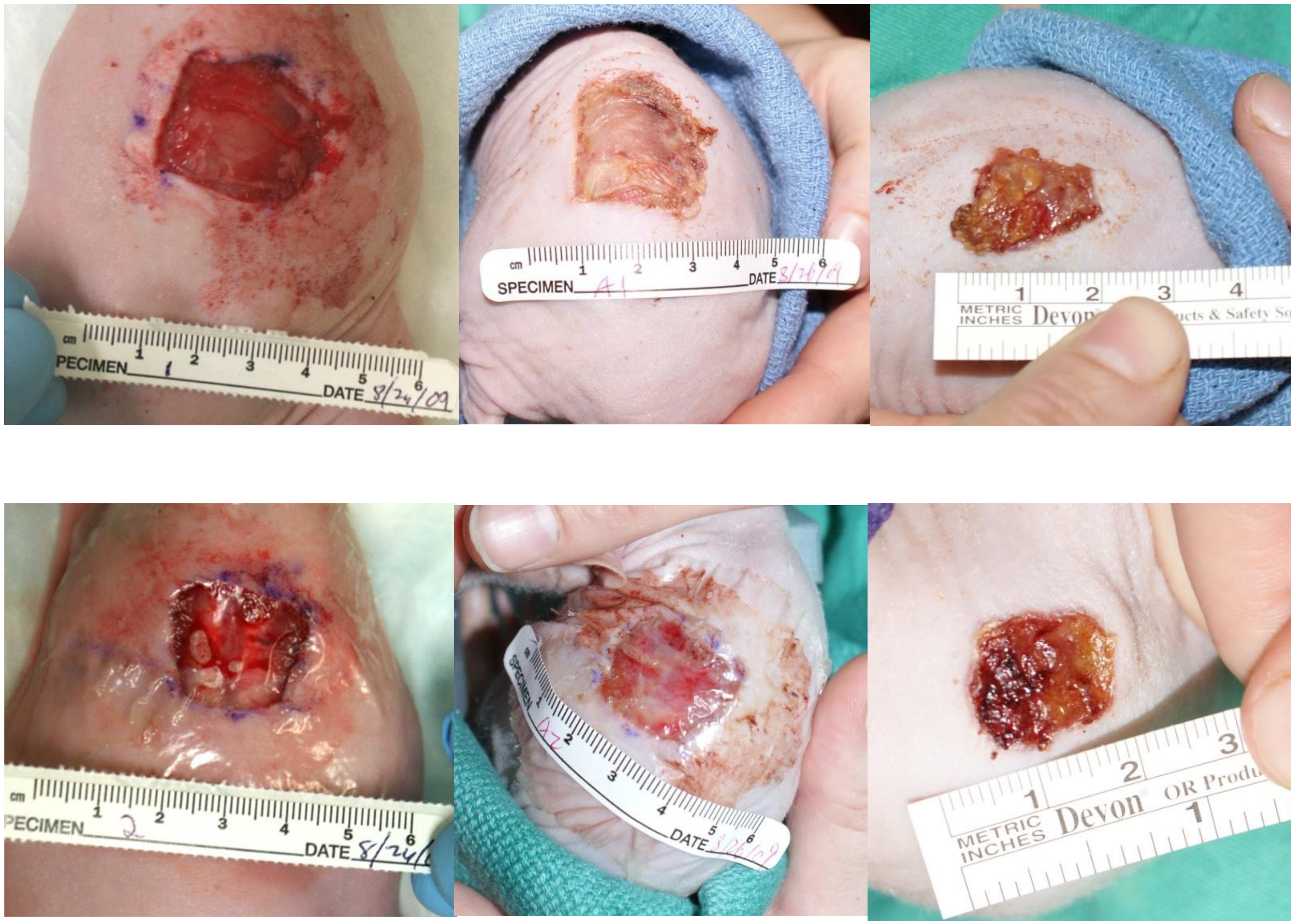


Wound Healing over Time

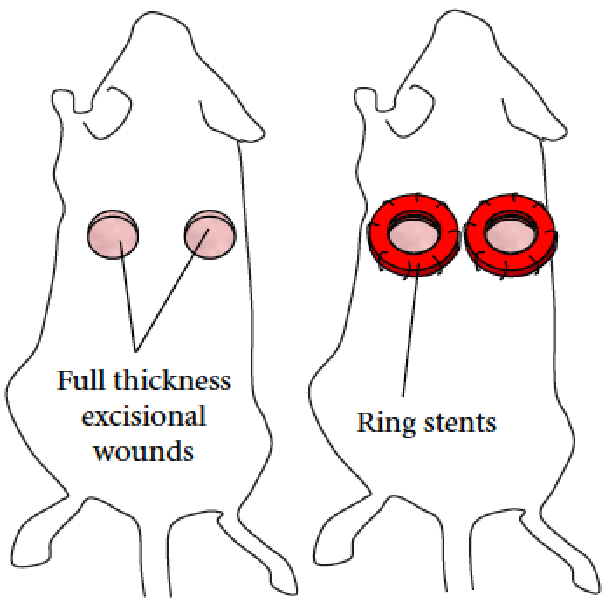
Day 0 | **Day 2** | **Day 8**

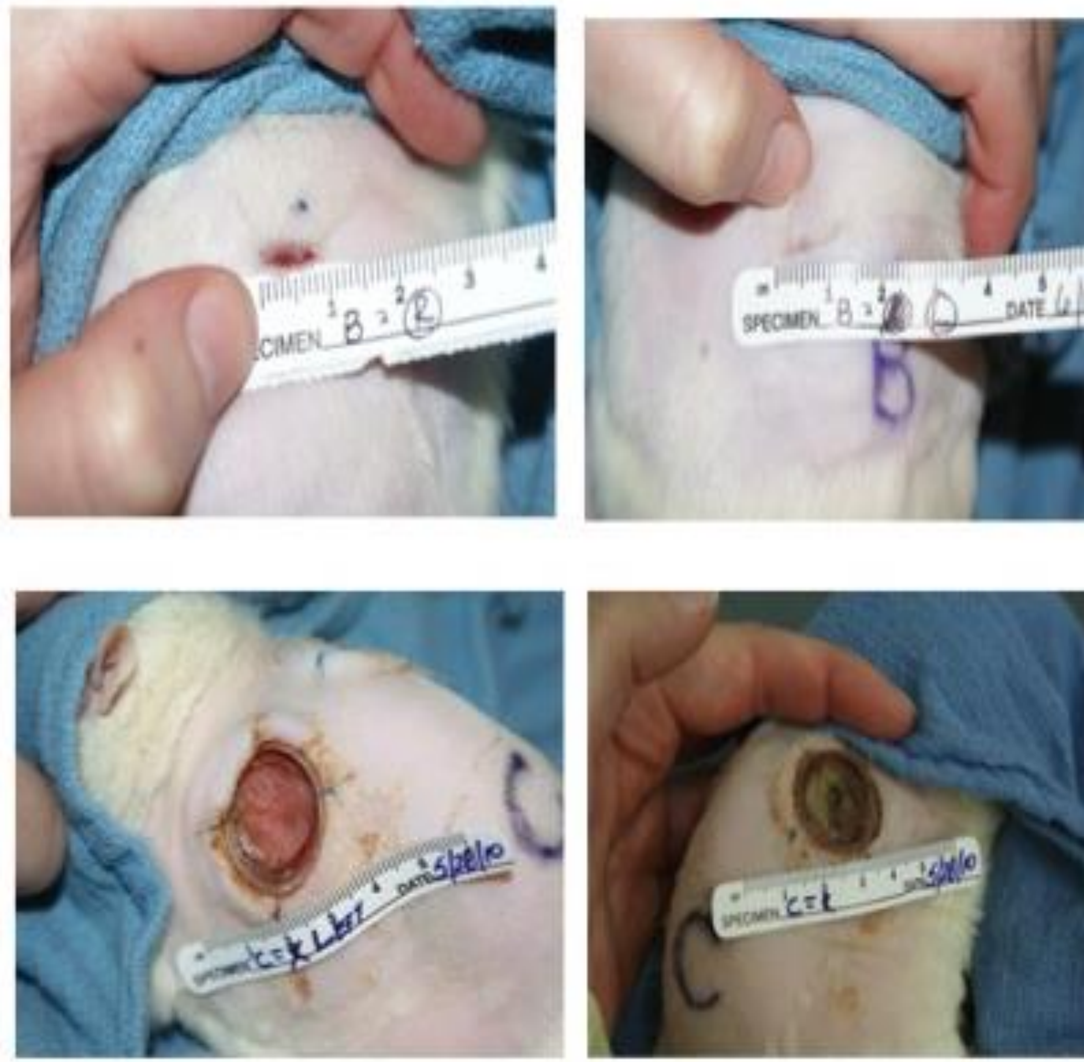
SPI/PEO Scaffold

Tegaderm (control)

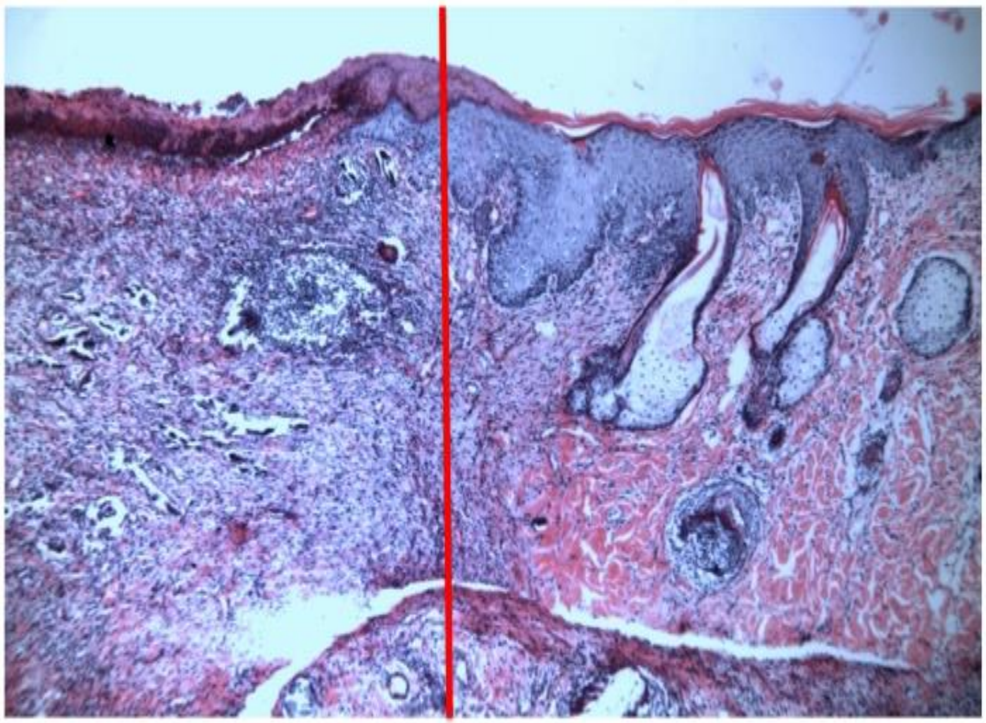


Rat Ring Model

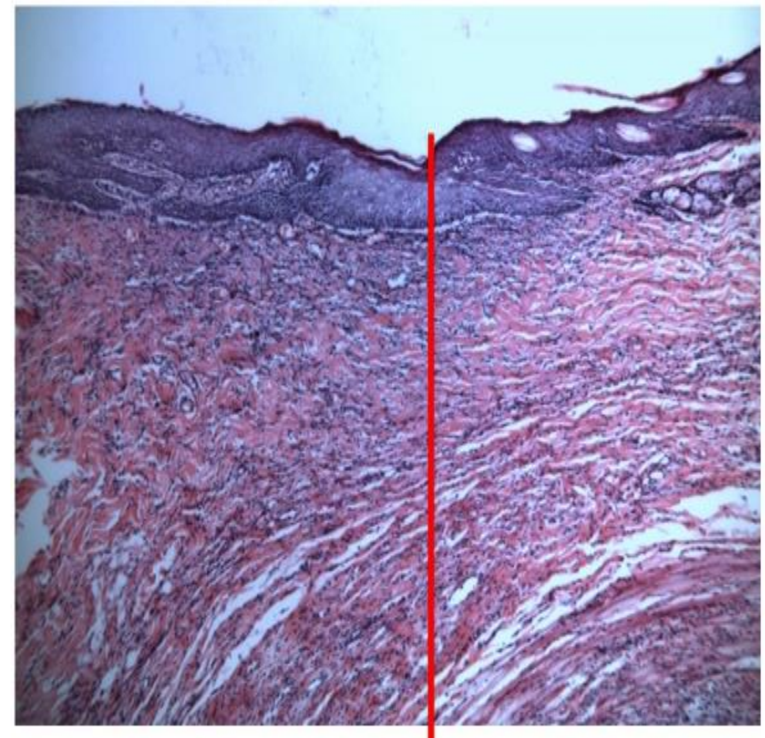




OmegaSkin™ Improves Re-Epithelialization in Rat Model

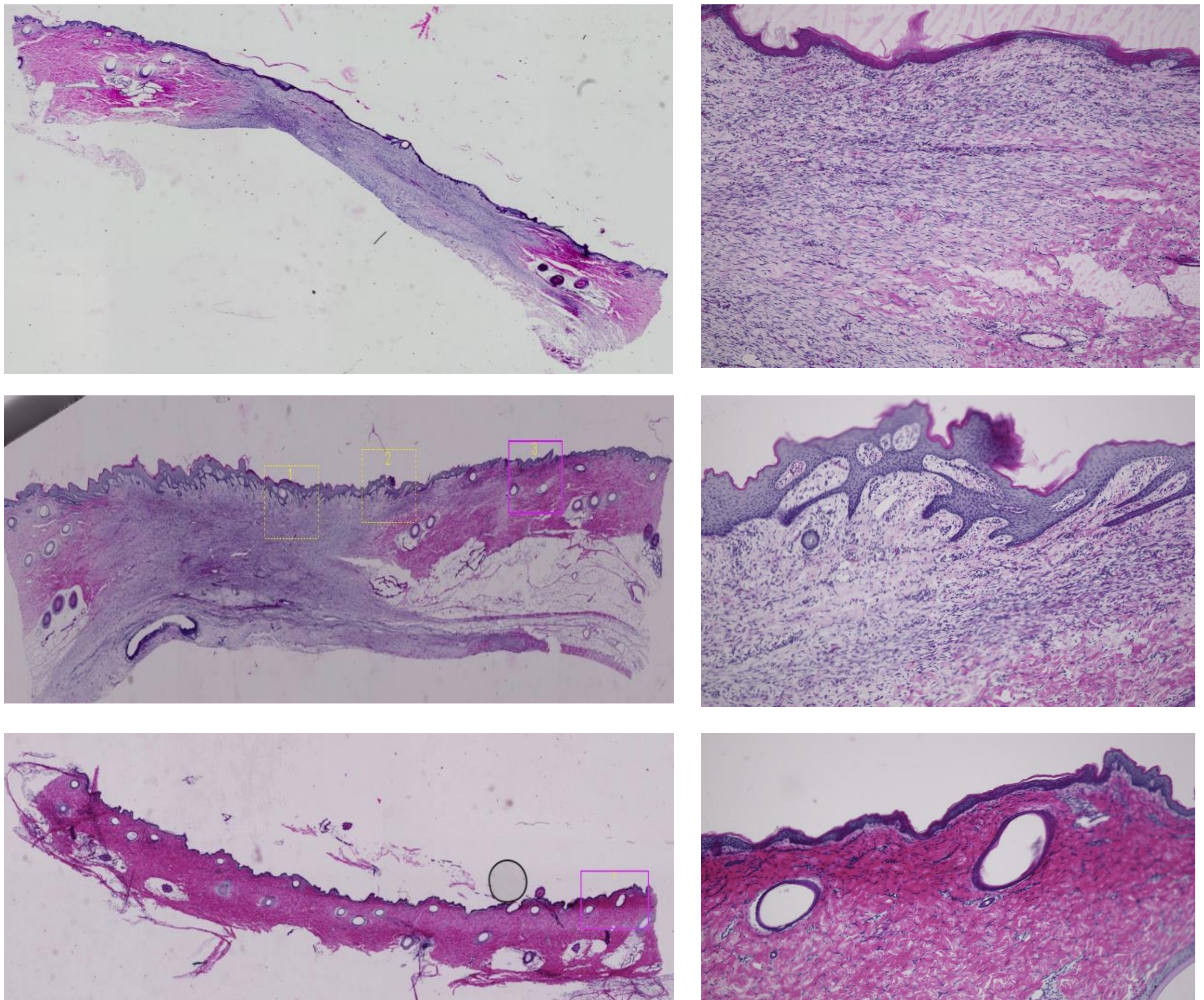


Wounded area Healthy border zone



Wound Healthy border

Histology: Improved Re-Epithelialization in Pig Model



Conclusions – Work in Progress

- Creation of Inexpensive Plant-Based Nanofibrous Bioactive, Biodegradable Wound Dressing
  - Feasibility and Efficacy tested in small (rat) and larger (pig) animal models
  - Enhance Epithelialization
  - Reduces Scarring
  - No Overt Immunoreaction
- Ongoing Mechanistic Research
- Licensed Product is Being Translated by Eqalix

Paths to Manufacturing and Commercialization of OmegaSkin™

- **Technology transfer:** Transfer gall relevant data, SOPs to Licensing Company (Eqalix).

- **Specifications:** Agreement on and “freeze” of an initial set of specifications for OmegaSkin™ that describe the product to be manufactured by third parties. Specifications for OmegaSkin™ include:
  – Fiber size in nanometers,
  – Density of mesh (i.e. pore size between fibers),
  – Bioassays for the assessment of biologic compatibility/attraction of fibroblasts,
  – Mechanical Properties(tensile strengths)
  – Chemical analyses (need to make sure that no solvents and reagents are left in the finished product that can cause a toxicology issue).
  – Shortlist of 4-6 specs that are easy starting points for an outsourced manufacturing effort

Paths to Manufacturing and Commercialization of OmegaSkin™

- **Manufacturing partners:** Identify potential manufacturing partners, , NDA and transfer of initial specs and the method for manufacturing scale-up prototype OmegaSkin.

- **Publications:** Timely, High Impact Journals

- **Investors:** Identify investors, and the availability of the publications MS is one of the factors for that meeting

- **Positioning:** Identify potential partners locally, nationally, internationally for distribution, once we have an approved 510k in the US. Positioning as "first industrial production of plant-based high-tech wound matrix" seems to resonate with interested parties because it could expand the market significantly and changes the price dynamics of existing approaches.

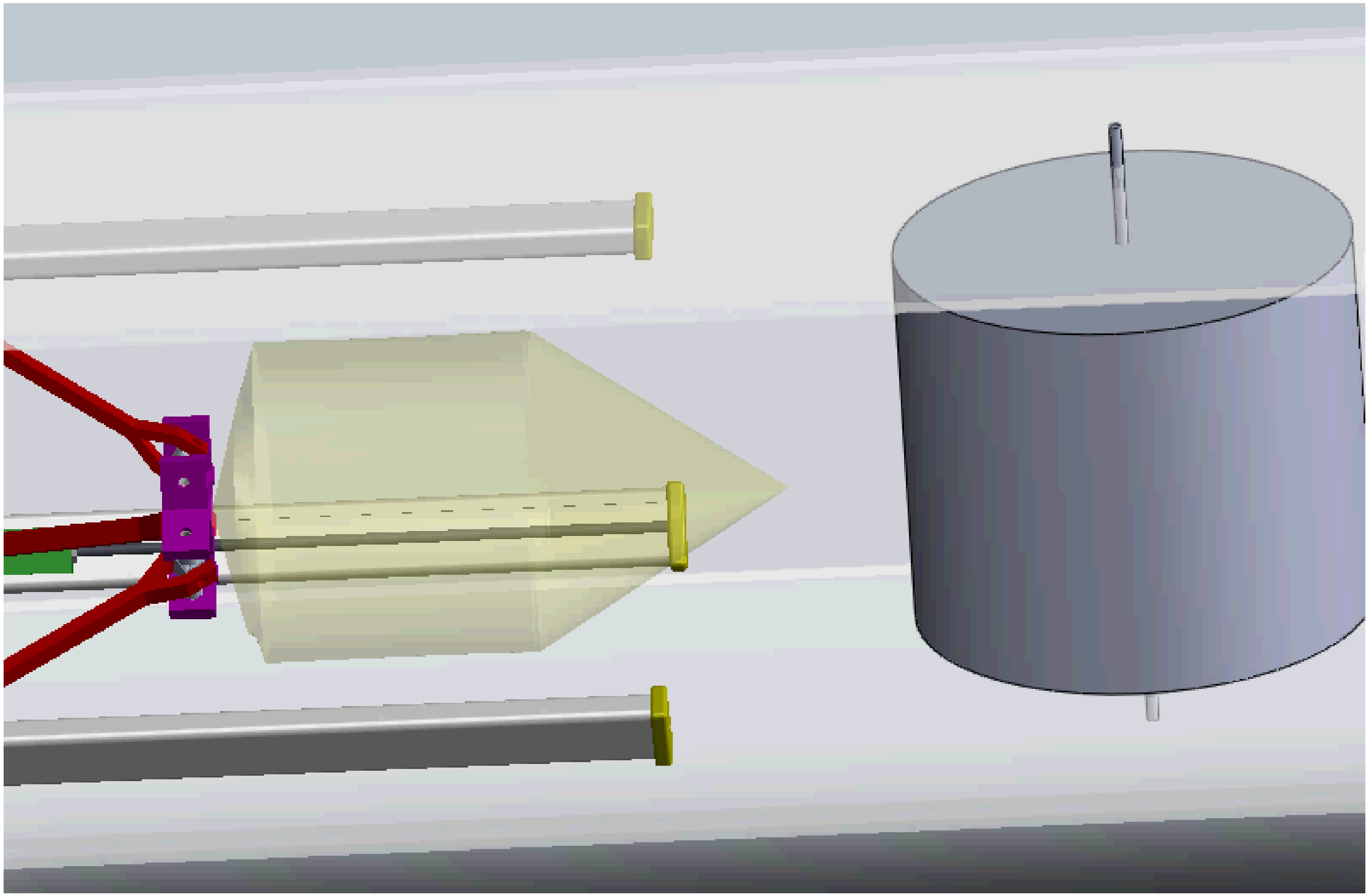
Paths to Manufacturing and Commercialization of OmegaSkin™

- **Goal: translating the process from R&D into commercial manufacturing**
  - Tech transfer: Transfer analytical methods, and validation under GLP
  - GLP Process scale up, optimization, qualification/validation as appropriate Stability study on representative batches (n=3)
  - GLP Manufacture of representative batches (n=3) for testing (in vivo, biocompatibility, whatever is required from a regulatory perspective
  - Transition to GMP - Manufacture of GMP batches for registration
  - Formal stability program on GMP batches
  - Establish Q/A oversight
  - Q/A audit of potential manufacturing partners: due diligence analysis of the manufacturing process , analytical characterization, and help think about translating the process from R&D into commercial manufacturing

Innovation: Electrospinning Robot

- Using a mobile spinneret, we can create larger OmegaSkin^{TM} sheets
- Use 3-D printer to build a 3-D printer for free-form nanofiber deposition in 2-D and 3-D:
- Needle can move freely in a 4.5x4.5x6 inch area
  - Traditional setup fabrics limited to 2x2 inches
  - Mobile head setup, limited to 5.5x5.5 inch fabric.
  - Using a moving collector as well (either a drum or a belt), fabrics as wide as 8.5 inches can be woven.
- Head can be driven 8 inches towards/away from target to optimize spinning distance, or spin onto awkwardly shaped collectors

Robot covering a 8.5 inch drum



Robot covering a 30 x 30 in^2 sheet