Visualization of Collagen Distribution in Skin by Means of Double Imaging by Two photon Excited Fluorescence and Second Harmonic Generation Implication for Developmental Biology

Motivation - considerable efforts of the scientific community worldwide are driven to describing the role of growth factors in regulation of tissue and organ development. Nevertheless, the mechanisms of initial activation or inactivation of particular growth factors as well as the primary causes of tissue inhomogeneity are still unclear.

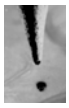
The present study is a continuation of a research carried out in the Groupe Matiere Condensee et Materiaux-UMR, by Vincent Fleury's group.

The aim - to study the effects of collagen orientation and distibution on mechanical "meso-scale" properties of normal tissue of chicken embryos during development in order to describe the role of mechanical stress in tissue development.

Methods

In vivo scanning tonometry

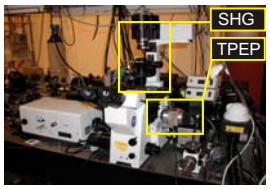
In vivo scanning tonometry setup: thin glass pipette (tip diameter 10-80μm) delivers the air flow to the sample, the spot of light reflected by the surface of the sample is detected by the camera, attached to the microscope



Principles of tonometry:

- Stiffness of tissues is evaluated on the basis of the size of the cavity created by an air flow on the tissue surface.
- The size of the cavity is deduced from the size of a spot of light, reflected from the surface and captured by a camera
- Stiffness is inversely proportional to the size of the cavity
- The measured parameter is the relative deformation of the tissue calculated as $Def=(B_{max}-B_{base})/B_{base}$, where B_{max} is the square area of the cavity, and B_{base} is the square area of the initial spot

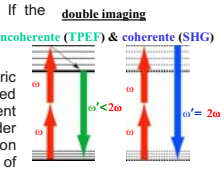
Two-photon excited fluorescence and 2-nd harmonic generation imaging



TPEF +SHG setup is based on the commercial confocal microscope Olympus IX71 +Fv300. The excitation light is supplied by titanium-sapphire laser, duration of impulse 200 fs, λ= 810 nm, power<100mW

Principles of TPEF +SHG imaging:

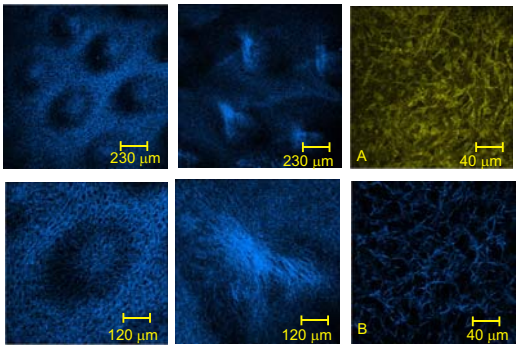
- TPEF arises from the simultaneous absorption of two photons in a single quantities event. The necessary power of light is supplied by focusing mode-locked (pulsed) lasers. The emitting light has a roughly doubled frequency compared to the incident light and is incoherent to it.
- SHG is a nonlinear optical effect induced by very intense light. If the intensity of light is sufficient, the induced polarization in a material is not linearly proportional to the incident electric field. Instead it can be expressed by a power series in the incident electric field. The second-order term in this nonlinear relation describes SHG, the formation of light with the doubled frequency of the incident light. The emitting light is coherent to the incident light.



Results

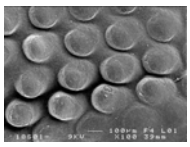
Collagen distribution and stiffness in feather buds & interbud area of the chicken embryo

Two photon-excitated fluorescence and second harmonic generation images of the chicken embryo skin dermis

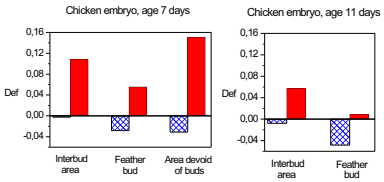


9 day old embryo, 11 day old embryo, 9 day old embryo, A-TPEP, B-SHG

- Collagen fiber distribution in dermis is detected by the SHG, while TPEF visualized fibroblasts.
- Areas of higher density of collagen correspond to the feather follicles and interbud area.



Feather buds and interbud areas of the skin of 9 day old chicken embryo pictured by scanning electron microscopy.



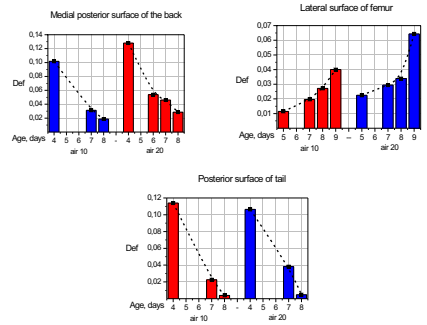
In vivo measurements of stiffness of the feather buds, interbud regions and areas devoid 4of buds:

The stress created by an air flow induces two types of deformation of the surface:

- positive deformation (increase of the spot size) corresponds to formation of cavity, stiffer surfaces make smaller cavities

- negative deformation (decrease of the spot size) reflects descending of the entire surface, due to its low ability to deform because of high stiffness

In vivo measurements of stiffness of different regions of the chicken embryo during their development



- Stiffness of the entire surface of the back as well as the posterior surface of the tail bud increases with age.
- Lateral surface of femur experiences a significant softening. This phenomenon is probably linked to rapid growth of the lower extremity observed in this period.
- The growth of the tissue is associated with intense synthesis of glycosaminoglycans in the connective tissue matrix, leading to an increase of the osmotic pressure. This leads to lowering of collagen concentration and as a consequence to decreasing of the tissue stiffness.

Conclusions

- Both in feather buds and interbud regions the fibers are ordered: radially in feather bud areas and linearly in the interbud areas
- The orientation of collagen fibers in the feather buds may promote growth of elongated structures from the center of the their radial distribution
- Different levels of stiffness in the feather buds and interbud regions may be caused not only by different concentrations of collagen in these regions but also by its different orientations.
- Lowering of stiffness in developing tissue is one of the key factors in the tissue development promoting free reproduction of cells and expansion of the organ volume. This effect may be realized both due to mechanical mechanisms and switching on of growth factors sensitive to the stress fields.

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