Physical parameters of 3D in vitro models of bone directing collagen orientation

Eemeli Vahteristo, Tuomas Näreoja Doc.,
Katerina Filatova M.Sc.
MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Introduction

The strength of bone is mainly determined by the orientation of its components. The largest contributor to the properties of bone is collagen produced by osteoblasts, or bone forming cells. Collagen can be deposited randomly around the osteoblasts or in concentric layers usually around capillary blood vessels (Figure 1C). The randomly oriented or woven bone (Figure 1A) is formed quickly but is not as strong or dense as the layered lamellar bone (Figure 1B). Being able to control the formation of lamellar bone with minimal amounts of woven bone would be important for medical research as it can help with bone prosthetic development.

Hypotheses

Following factors can affect the orientation of forming collagen:

- **Fluid flow**
- **Physical stress**
- **Existing collagen network**

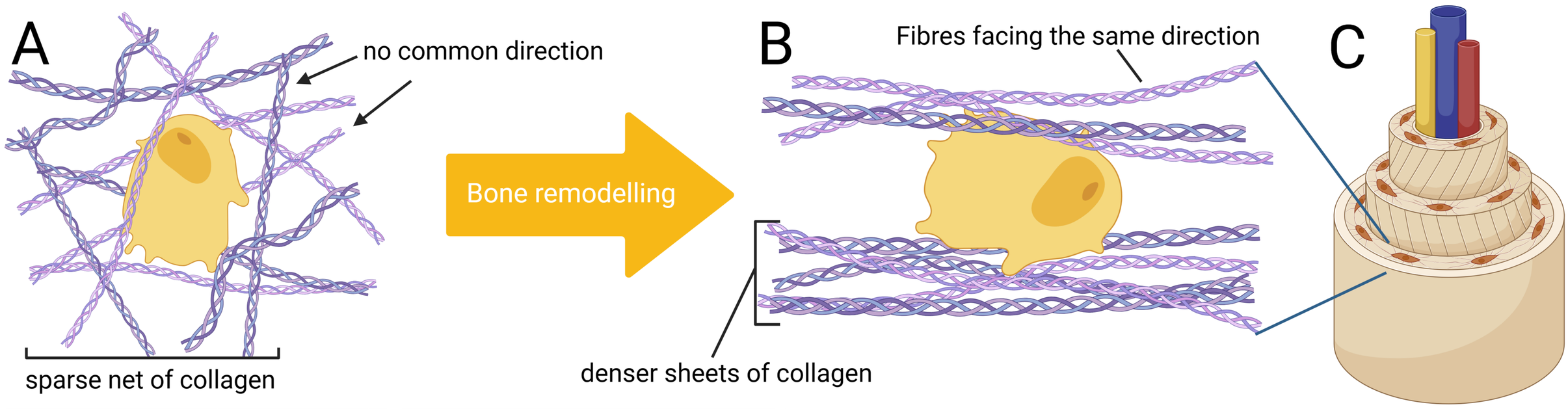

Figure 1. Difference between woven and lamellar bone. A: Illustration of woven bone B: illustration of lamellar bone C: Osteon with concentric layers around a bundle of blood vessels. Woven bone is randomly oriented and weak while lamellar bone is formed from dense sheets called lamellae. Woven bone is slowly broken down and remodelled so all bone is comprised of lamellar bone in a healthy adult skeleton.

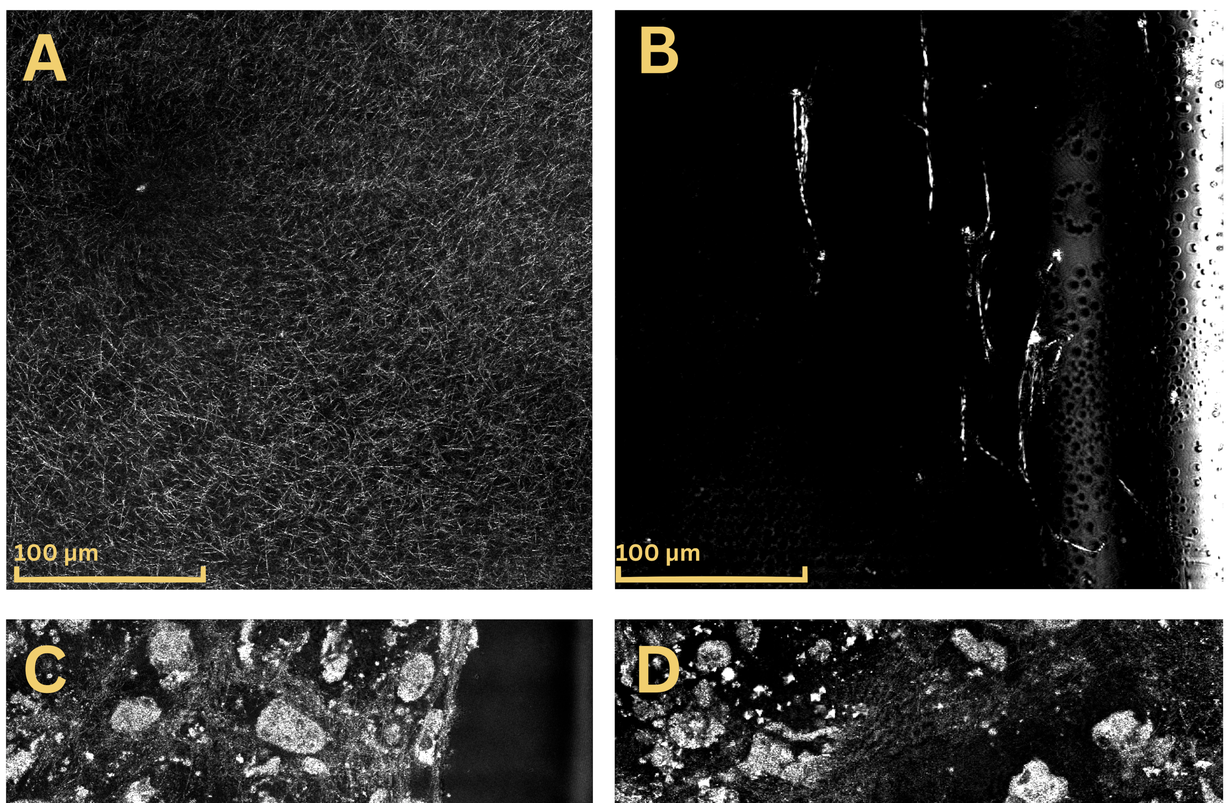

Figure 2. Collagen in different forms. A: Unmodified collagen hydrogel. B: collagen nucleated under flow (2D). C & D: cell mediated collagen formation (2D). Flow was applied vertically in all samples. Images A and D show unordered collagen showing short fibres without a general direction. Images B and C on the other hand show longer strands of collagen oriented along the flow. Directionality is measured as probability density in an area. While both Image C and D were both taken from the same sample, Image C contains clear directionality in its collagen network while Image D has largely unorganised collagen. The edge of the slide in Image C may have influenced the flow to create strong flow-wise orientation.

Acknowledgements: Imaging was performed at the Cell Imaging and Cytometry Core, Turku Bioscience Centre, Turku, Finland, with the support of Biocenter Finland. Illustrations were made using BioRender

Materials and methods

MC3T3-E1 mouse pre-osteoblasts were grown under oscillatory laminar flow to study the effect of liquid shear stress on the orientation of the resulting extracellular matrix. To study newly forming bone, osteoblasts were grown in a flow chamber (Luer III 3in1, ibidi) without external matrices under a defined shear stress of 15 dyn/cm2. In studying bone remodeling, larger 3D flow chambers (Luer I 3D, ibidi) with an existing collagen hydrogel was employed as a base for the osteoblasts where shear stresses of 0, 5, 15 and 30 dyn/cm2 were used. Samples were imaged with Leica TCS SP5 confocal microscope utilizing backscattered light from collagen.

Results and Future

2D sample culturing and fluorescence imaging has concluded with little discernible difference in collagen directionality between flow treated and control samples. This find could hint towards the cells requiring an existing collagen network and lamellar bone formation only happening through bone remodelling. The study is still ongoing, additional imaging with specific stains for collagen and hydroxyapatite as well as alkaline phosphatase assays will be conducted. Other tissue types such as simulated capillary blood vessels could be introduced to the samples to study the natural osteon formation.