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**PURPOSE:** Coordination of bone formation and resorption is necessary for the success of bone regenerative strategies. Characterization of the instructive capabilities of extracellular matrix (ECM)-inspired materials for osteoprogenitor differentiation has sparked questions on the interactions between such materials and the host microenvironment. Previously, we demonstrated that a nanoparticulate mineralized collagen glycosaminoglycan (MC-GAG) scaffold is both highly osteogenic without the need for exogenous growth factor stimulation and induces secretion of osteoprotegerin (OPG), an endogenous decoy receptor against the receptor activator of nuclear factor-κB ligand (RANKL), a necessary osteoclastogenic factor. In this work, we combine an adenoviral mediated expression of OPG (AdOPG) in primary human mesenchymal stem cells (hMSCs) with MC-GAG to understand the role for osteoclast inactivation in augmentation of bone regeneration.

**METHODS:** Control and AdOPG transduced primary hMSCs were cultured on Col-GAG or MC-GAG materials in osteogenic differentiation medium. OPG and RANKL expression were evaluated using quantitative reverse transcriptase polymerase chain reaction (QPCR), western blot analysis, and enzyme linked immunosorbent assay (ELISA). Co-cultures of control and AdOPG transduced hMSCs on Col-GAG and MC-GAG with primary human osteoclasts were performed. Osteogenic differentiation was evaluated with western blot, ELISA, and micro-computed tomography for osteogenic differentiation. Simultaneously osteoclast activity was assessed with tartrate resistant acid phosphatase staining and resorption pit assays.

**RESULTS:** hMSCs differentiated on MC-GAG expressed a lower ratio of endogenous RANKL/OPG protein compared to a non-mineralized collagen glycosaminoglycan (Col-GAG) scaffold. In both materials, the RANKL/OPG ratio was further lowered significantly in the presence of AdOPG compared to control hMSCs without significant difference between the materials. We established a co-culture system to understand the interplay between differentiating hMSCs on Col-GAG or MC-GAG and differentiating human primary pre-osteoclasts. Control hMSCs on Col-GAG or MC-GAG in co-cultures did not differ in viability or proliferation compared to each other or to hMSC single cultures. However, AdOPG-transduced hMSCs on MC-GAG was modestly decreased in viability or proliferation compared to the Col-GAG counterpart in co-cultures. Co-cultures with differentiating osteoclasts increased hMSC mineralization with or without AdOPG, particularly in MC-GAG. In contrast, the viability and proliferation of osteoclasts in co-culture were significantly decreased in the presence of AdOPG-transduced hMSCs. While co-culture with control hMSCs on either Col-GAG or MC-GAG upregulated the resorptive activity of osteoclasts, AdOPG-transduced hMSCs reduced the resorption with a greater effect on MC-GAG compared to Col-GAG.

**CONCLUSION:** The addition of osteoprotegerin to MC-GAG-mediated hMSC osteogenic differentiation simultaneously diminishes osteoclast resorptive capacity without affecting the positive regulatory effects on osteogenic differentiation.

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**Inferring 3D Craniofacial Skeletal Shape from Facial Surface Geometry Using Reverse Engineering of a Forensic Tissue Depth Model**

**Presenter:** Zachary Fishman, P.Eng, MASc

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**PURPOSE:** The face and craniofacial skeleton (CFS) make up a complex 3D structure that is critical to human function and cosmesis. Traumatic injury to the CFS requires fracture treatment to both allow the recovery of mechanical function and forms a foundation for the restoration of soft tissue anatomy. CFS reconstruction aims to restore pre-injury appearance, however in severe injuries shape information for the skull and facial bones may be missing. This presents a particular challenge in bi-frontal injuries and pan-facial fractures where the mirror imaging of the intact side of the head cannot be used to guide reconstruction.
The reconstruction of 3D facial surface geometry from pre-injury 2D photographs has recently been established through large scale morphable face modeling. As well, in forensic sciences, models with variable soft-tissue depths are used to determine face shape from skull geometry. This study aims to ‘reverse-engineer’ a forensics’ tissue depth model to determine pre-injury CFS shape from reconstructed 3D facial geometry. It is hypothesized that 3D forensics data can be used to fill in missing gaps in CFS geometry with sufficient accuracy to guide pre-operative planning for CFS reconstruction.

**METHODS:** The forensics’ tissue depth model was applied to 3D facial geometries acquired through segmentation of head CT data. Age, sex and BMI were used as input parameters to guide the application of the forensics’ tissue depth model data to each face. The tissue depths between the face and CFS were determined by finding the Euclidean distance transform (nearest neighbor) employed by the original forensics study and via calculation using normal vectors generated from the face surface. Calculated tissue depth was evaluated against measured thickness on the head CT between the segmented CFS (bone) and the skin.

**RESULTS:** Tissue depth determined by nearest neighbor and normal vector measurements yielded accurate reconstructions of the frontal and zygoma bones (within 1mm, or +/- 2 voxels). However, only the normal vector technique succeeded in estimating tissue depth in bone regions where the face and skull have differing concavity (i.e. eye sockets, maxilla). Agreement was more limited in the lower facial skeleton where greater variation of soft tissue structures occur.

**CONCLUSION:** The reversed forensics tissue depth model was found to appropriately infer bony anatomy for the upper CFS from 3D face geometry. The 3D skull shaping provided by this work yields sufficient accuracy to warrant its inclusion into a translational pipeline of tools for pre-operative planning for CFS reconstruction.

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**Microsurgery for Parotidectomy Defects: A Powerful and Versatile Tool for Aesthetic and Functional Reconstruction**

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**PURPOSE:** Surgical management of parotid pathologies may lead to heterogeneous defects with variable involvement of skin, soft tissue, and bone along with possible sacrifice of the facial nerve. Local tissues are often inadequate to address these diverse reconstructive needs. We therefore aim to evaluate the role of microsurgery in reconstruction of parotidectomy defects.

**METHODS:** All microsurgical reconstructions for parotidectomy defects performed were identified and reviewed. Patient demographics, intra-operative variables including microsurgical flap characteristics, and reconstructive outcomes were analyzed.

**RESULTS:** A total of 11 microsurgical reconstructions in ten patients were performed. Average patient age and body-mass index were 38.5 years and 25.94 kg/m². Six patients (60.0%) had a former smoking history while three (30.0%) had major medical co-morbidities. Six patients (60.0%) had prior surgical intervention with prior partial parotidectomy/enucleation (30.0%) being most common. Two patients each (20.0%) had undergone prior radiation and chemotherapy. Five (50.0%) and three (30.0%) patients underwent adjuvant radiation and chemotherapy, respectively. Average follow-up was 116.4 days.

The most common tumor pathologies were pleomorphic adenoma, acinic cell carcinoma, and squamous cell carcinoma (20.0%, each). Primary surgical procedures included total parotidectomy (30.0%), superficial parotidectomy (30.0%), radial parotidectomy (20.0%), and revision parotidectomy (20.0%). Four (40.0%) and two (20.0%) patients underwent concurrent neck dissection and bony resections. Three patients (30.0%) had facial nerve branch sacrifice reconstructed with nerve grafts in three cases (100.0%) and nerve transfers in two cases (66.7%).

Microsurgical free flaps utilized included medial sural artery perforator (MSAP) flaps in six patients (60.0%) and anterolateral thigh (ALT) flaps in five (50.0%) patients. Average flap and skin paddle sizes were 79.61 cm² and 5.33 cm², respectively. Two flaps (18.2%) were completely de-epithelialized and buried. Ten donor sites (90.9%) were