1- Bone Marrow Cells are a Source of Undifferentiated Cells to Prevent Sjögren's Syndrome and to Preserve Salivary Glands Function in the Non-Obese Diabetic Mice

Non-obese diabetic (NOD) mice develop Sjögren's-like syndrome (Ss) and a gradual loss of saliva secretory function. Our previous study showed that injections of matched normal spleen cells with Complete Freund's Adjuvant (CFA) reversed salivary gland dysfunction in 14-week-old NOD mice, which had established Ss. The spleen and bone marrow are closely related organs, and both are among the first sites of hematopoiesis during gestation. Noticing a rapidly increasing number of clinical trials using bone marrow (BM) cells treatments for autoimmune diseases, we tested if BM cells can prevent Ss and restore salivary glands' function. We injected CFA and MHC class I-matched normal BM cells in 7-week-old NOD mice, which had not yet developed Ss. We found at week 52 post-treatment that all NOD mice receiving BM cells and CFA had a recovery of salivary flow and were protected from Ss and diabetes. BM cells treated mice had their salivary function restored quantitatively and qualitatively. Saliva flow was higher (p < 0.05) in BM cells-transplanted mice when compared to control mice, which continued to deteriorate over time. Total proteins, epidermal growth factor, amylase, and electrolytes concentrations in saliva of BM cells-treated mice were not significantly changed at week 44 and 52 post-therapy when compared to pre-therapy (when the mice did not have Ss). Restoration of salivary flow could have resulted from a combination of rescue and paracrine effects from BM cells. This study suggests that a combined immunoand cell-based therapy can permanently prevent Ss and restored salivary function in NOD mice.

2- Cells from Bone Marrow that Evolve Into Oral Tissues and Their Clinical Applications

There are two major well-characterized populations of post-natal (adult) stem cells in bone marrow: hematopoietic stem cells which give rise to blood cells of all lineages, and mesenchymal stem cells which give rise to osteoblasts, adipocytes, and fibroblasts. For the past 50 years, strict rules were taught governing developmental biology. However, recently, numerous studies have emerged from researchers in different fields suggesting the unthinkable that stem cells isolated from a variety of organs are capable of ignoring their cell lineage boundaries and exhibiting more plasticity in their fates. Plasticity is defined as the ability of post-natal (tissue-specific adult) stem cells to differentiate into mature and functional cells of the same or of a different germ layer of origin. There are reports that bone marrow stem cells can evolve into cells of all dermal lineages, such as hepatocytes, skeletal myocytes, cardiomyocytes, neural, endothelial, epithelial, and even endocrine cells. These findings
promise significant therapeutic implications for regenerative medicine. This article will review recent reports of bone marrow cells that have the ability to evolve or differentiate into oral and craniofacial tissues, such as the periodontal ligament, alveolar bone, condyle, tooth, bone around dental and facial implants, and oral mucosa. Oral Diseases (2007) 13, 11–16

3- Distribution of Tight Junction Proteins in Adult Human Salivary Glands

Tight junctions (TJs) are an essential structure of fluid-secreting cells, such as those in salivary glands. Three major families of integral membrane proteins have been identified as components of the TJ: claudins, occludin, and junctional adhesion molecules (JAMs), plus the cytosolic protein zonula occludens (ZO). We have been working to develop an orally implantable artificial salivary gland that would be suitable for treating patients lacking salivary parenchymal tissue. To date, little is known about the distribution of TJ proteins in adult human salivary cells and thus what key molecular components might be desirable for the cellular component of an artificial salivary gland device. Therefore, the aim of this study was to determine the distribution of TJ proteins in human salivary glands. Salivary gland samples were obtained from 10 patients. Frozen and formalin-fixed paraffin-embedded sections were stained using IHC methods. Claudin-1 was expressed in ductal, endothelial, and ?25% of serous cells. Claudins-2, -3, and -4 and JAM-A were expressed in both ductal and acinar cells, whereas claudin-5 was expressed only in endothelial cells. Occludin and ZO-1 were expressed in acinar, ductal, and endothelial cells. These results provide new information on TJ proteins in two major human salivary glands and should serve as a reference for future studies to assess the presence of appropriate TJ proteins in a tissue-engineered human salivary gland. (J Histochem Cytochem 56:1093–1098, 2008)

4- Apoptotic Effect of Different Self-Etch Dental Adhesives on Odontoblasts in Cell Cultures

Objectives: we aimed to evaluate the potential cytotoxicity (apoptosis-induction) of three types of self-etch dental adhesives; two-component one-step (Xeno III), two-components two-steps (Clearfil Protect Bond) and one-component one-step (Xeno V) on cultured odontoblasts. Methods: Each adhesive was prepared to simulate its clinical manipulation. Cured sterile individual masses were immersed in DMEM and left at 37°C for 24 hours. Then a volume of 100 &frac14; L of the extract medium was added to the cultured odontoblasts and incubated for additional 24 hours, 48 hours and 72 hours, respectively. Acridine orange-propidium iodide (AO-PI) labeling was employed to assess the proportion of dead to total number of cells. In addition, an in situ apoptosis detection kit was used to evaluate the DNA cleavage and chromatin condensation employing the immunohistochemical (IHC) technique. Statistical analysis of the data was performed using one-way ANOVA and. Results: Both apoptosis evaluation methods revealed comparable results with the exception that IHC showed 5-7% less number of dead cells when compared to similar groups evaluated by AO-PI. The percentages of dead to total cells after treatment with Clearfil Protect Bond, Xeno III and Xeno V, were significantly
different from the percentage of dead cells after treatment with DMEM alone (-ve control), P value < 0.05 and Xeno V dental adhesive had the weakest cytotoxic effect on odontoblasts followed by Xeno III especially after 24 hours of incubation. Clearfil Protect Bond had the strongest cytotoxic effect on odontoblasts that was almost closer to that of staurosporine in DMEM (+ve control). Conclusion: All tested dental adhesives had remarkable adverse effect on the odontoblasts in vitro; this might be of concern when applied clinically in deep cavities where such cytotoxic chemicals become in close contact to dental pulp. Therefore, further in vivo studies on animal models are recommended to support or refute these in vitro findings.

5-

**Cell Surface Markers CD44 and CD166 Localized Specific Populations of Salivary Acinar Cells**

**OBJECTIVE:**

Experimental approaches tested to date for functional restoration of salivary glands (SGs) are tissue engineering, gene transfer, and cell therapy. To further develop these therapies, identifying specific cell surface markers for the isolation of salivary acinar cells is needed. To test a panel of cell surface markers [used in the isolation of mesenchymal stem cells, (MSCs)] for the localization of salivary acinar cells.

**MATERIALS:**

Human submandibular and parotid glands were immunostained with a panel of MSC markers and co-localized with salivary acinar cell differentiation markers [α±-amylase, Na-K-2Cl cotransporter-1, aquaporin-5 (AQP5)]. Additional cell markers were also used, such as α±-smooth muscle actin (to identify myoepithelial cells), cytokeratin-5 (basal ductal cells), and c-Kit (progenitor cells).

**RESULTS:**

CD44 identified serous acini, while CD166 identified mucous acini. Cytokeratin-5 identified basal duct cells and 50% of myoepithelial cells. None of the remaining cell surface markers (Stro-1, CD90, CD106, CD105, CD146, CD19, CD45, and c-Kit) were expressed in any human salivary cell.

**CONCLUSIONS:**

CD44 and CD166 localized human salivary serous and mucous acinar cells, respectively. These two cell surface markers will be useful in the isolation of specific populations of salivary acinar cells.

6-

**Matrigel Improves Functional Properties of Human Submandibular Gland Cell Line**

Sjogren’s syndrome and radiotherapy for head and neck cancers result in irreversible damage to functional salivary tissue, for which no adequate treatment is available. The
microenvironment for salivary gland cell cytodifferentiation is critical for the future development of salivary gland regeneration, repair and tissue engineering treatments. Results from this study indicate that human submandibular cell line (HSG) cultured on Matrigel (2mg/ml) could be induced to differentiate into polarized secretory acinar-like cells. The HSG cells grown on Matrigel were evaluated by physiological functional assays, molecular and immunohistochemistry, immunofluorescence, and morphological assessments. The results showed (1) a decrease in cell proliferation; (2) an increase in cell apoptosis; (3) cellular polarization evident by transepithelial electrical resistance (TER), expressions of tight junction proteins (claudin-1, -2, -3, -4, occludin, JAM-A, and ZO-1) and transmission electron microscopy (TEM); (4) an increase in the production and/or secretion of acinar cell proteins, i.e., alpha-amylase, aquaporin-5, cytokeratins, and mucin-1, that were not associated with increases in mRNA transcription; (5) a decrease in vimentin expression; and (6) expression of potential stem cell biomarkers CD44 and CD166. The data indicated that Matrigel provided a suitable microenvironment for morphological and functional differentiation of HSG cells into 3D acinar like cells. This study provides an in vitro model and baseline data on future developments of new strategies for salivary gland regeneration and replacement.

Matrigel Improves Functional Properties of Primary Human Salivary Gland Cells

Currently, there is no effective treatment available to patients with irreversible loss of functional salivary acini caused by Sjogren’s syndrome or after radiotherapy for head and neck cancer. A tissue-engineered artificial salivary gland would help these patients. The graft cells for this device must establish tight junctions in addition to being of fluid-secretory nature. This study analyzed a graft source from human salivary glands (huSG) cultured on Matrigel. Cells were obtained from parotid and submandibular glands, expanded in vitro, and then plated on either Matrigel-coated (2 mg/mL) or uncoated culture dish. Immunohistochemistry, transmission electron microscopy, quantitative real-time-polymerase chain reaction, Western blot, and transepithelial electrical resistance were employed. On Matrigel, huSG cells adopted an acinar phenotype by forming three-dimensional acinar-like units (within 24 h of plating) as well as a monolayer of cells. On uncoated surfaces (plastic), huSG cells only formed monolayers of ductal cells. Both types of culture conditions allowed huSG cells to express tight junction proteins (claudin-1, -2, -3, -4; occludin; JAM-A; and ZO-1) and adequate transepithelial electrical resistance. Importantly, 99% of huSG cells on Matrigel expressed α+-amylase and the water channel protein Aquaporin-5, as compared to

Human Mesenchymal Stem Cells Cultured with Salivary Gland Biopsies Adopt an Epithelial Phenotype

Sjogren’s syndrome and radiotherapy for head and neck cancer result in severe xerostomia and irreversible salivary gland damage for which no effective treatment is currently available. Cell culture methods of primary human salivary gland epithelial cells (huSGs) are slow and cannot provide a sufficient number of cells. In addition, the majority of cultured huSGs are of a ductal phenotype and thus not fluid/saliva secretory cells. Some reports indicated that mesenchymal stem cells (MSCs) possessed the potential to differentiate into epithelial cells. To test this hypothesis with huSGs, a
Coculture system containing 2 chambers separated by a polyester membrane was used to study the capacity of human MSCs to adopt an epithelial phenotype when cocultured with human salivary gland biopsies. Results were that 20%-40% of cocultured MSCs expressed tight junction proteins [claudin-1 (CLDN-1), -2, -3, and -4; occludin; junctional adhesion molecule-A; and zonula occludens-1] as well as other epithelial markers [aquaporin-5, \( x \)-amylose (\( x \)-AMY), and E-cadherin], and generated a higher transepithelial electrical resistance. Electron microscopy demonstrated that these MSCs had comparable cellular structures to huSGs, such as tight junction structures and numerous secretory granules. Quantitative real time (RT)-polymerase chain reaction revealed an upregulation of several salivary genes (aquaporin-5, AMY, and CLDN-2). Moreover, the amounts of \( x \)-AMY detected in cocultured MSCs were comparable to those detected in huSGs control cultures. These data suggest that cocultured MSCs can demonstrate a temporary change into a salivary gland acinar phenotype.

Bone Marrow-Derived Cells Rescue Salivary Gland Function in Mice with Head and Neck Irradiation

Treatment for most patients with head and neck cancers includes ionizing radiation. A consequence of this treatment is irreversible damage to salivary glands (SGs), which is accompanied by a loss of fluid-secreting acinar-cells and a considerable decrease of saliva output. While there are currently no adequate conventional treatments for this condition, cell-based therapies are receiving increasing attention to regenerate SGs. In this study, we investigated whether bone marrow-derived cells (BMDCs) can differentiate into salivary epithelial cells and restore SG function in head and neck irradiated mice. BMDCs from male mice were transplanted into the tail-vein of 18Gy-irradiated female mice. Salivary output was increased in mice that received BMDCs transplantation at week 8 and 24 post-irradiation. At 24 weeks after irradiation (IR), harvested SGs (submandibular and parotid glands) of BMDC-treated mice had greater weights than those of non-treated mice. Histological analysis shows that SGs of treated mice demonstrated an increased level of tissue regenerative activity such as blood vessel formation and cell proliferation, while apoptotic activity was increased in non-transplanted mice. The expression of stem cell markers (Sea-1 or c-kit) was detected in BMDC-treated SGs. Finally, we detected an increased ratio of acinar-cell area and approximately 9% of Y-chromosome-positive (donor-derived) salivary epithelial cells in BMDC-treated mice. We propose here that cell therapy using BMDCs can rescue the functional damage of irradiated SGs by direct differentiation of donor BMDCs into salivary epithelial cells.