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Evolving technologies in regenerative medicine

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Abstract:

This short review explores the constantly evolving technology, both cellular and equipment or technology based, which is currently being experienced in the field of regenerative medicine. There is considerable ongoing research into stem cell technologies but there is currently a translational block in bringing some of these technologies to the clinic. We propose new and evolving technologies which may resolve this cell-based block and quickly bring regenerative medicine technologies safely and effectively to patients in need.

Introduction

Regenerative medicine (excluding routine bone marrow transplantation) has developed over the past 20-35 years based on concepts around stem cells[1]. This has been on the premise that stem cells are self-replicating and can therefore potentially repair and restore damaged or diseased tissue, for example during wound healing[2]. Many different stem cells, potentially useful in regenerative medicine, have been identified in almost every tissue in the body. These include Haemopoietic Stem Cells (HSC)[3-6], Mesenchymal Stem Cells (MSC) from various sources[7,8], Embryonic Stem Cells[9,10] and induced Pluripotent Stem Cells (iPSC)[11,12]. Despite this extensive amount of literature on stem cell technology (some of it in the most highly regarded journals) there are currently no stem cell therapies (apart from HSC transplantation for haematological malignancy) in current routine clinical practice. There seems to be a block at the translational stage[13] perhaps related to stringent regulatory requirements[14]. In this short review, we will present some emerging and evolving technologies which may help to bring much needed help to patients needing regenerative medicine procedures.

Extracorporeal Blood Oxygenation and Ozonation (EBOO)

EBOO therapy is an emerging technology which has already been used by some clinicians and appears to be a safe and effective option for some patients[15]. There has been considerable progress with the use of Ozone therapy in the treatment of Covid19 both in the acute stage and in 'long Covid'[16-19]. The use of ozone therapy has recently been described in the safe and effective treatment of musculoskeletal diseases such as rheumatoid arthritis[20,21], osteoarthritis[22], herniated disc[23] and temporomandibular joint disorder[24]. Other workers have described considerable the benefit of EBOO in the treatment of peripheral arterial disease, coronary disease, severe dyslipidaemia, coronary disease, Madelung disease and deafness of vascular origin[25]. The current data on the use of EBOO in peripheral artery disease (PAD) seem especially encouraging with no side effects or complications being reported[26]. These authors conclude that EBOO is an effective treatment for skin lesions in PAD along with additional benefits to general health and wellbeing. There was, however, no observed change in arterial circulation in these patients suggesting that the

mechanism of action of EBOO is complex and still to be fully understood. There has also been considerable progress in the use of EBOO in the potentially deadly disease of necrotizing fasciitis[27]. This treatment approach using EBOO is now used in many hospitals as a routine treatment of necrotizing fasciitis. EBOO clearly has a wide range of very beneficial and safe therapeutic use, this makes EBBO an important contributor to the overall holistic patient treatment in parallel to regenerative medicine procedures[28]. Our own experience with EBOO at RegMedGoergia is equally promising (with publications in preparation) and there is no doubt that EBOO will make a very important contribution to the overall practice of regenerative medicine in the future.

Photomodulation

Photomodulation (sometimes known as photobiomodulation) has been shown the be a useful process in the optimisation of regenerative medicine procedures involving MSC[29]. Low energy laser light has also been shown to be effective in the activation of adipose derived stem cells[30]. It is clear that photomodulation is a useful tool for the spatiotemporal modulation of a wide range of biological systems where photoresponsive components are present[31]. Photomodulation can be used in two main ways[32]:

1. Photomodulation of a therapeutic vehicle or medication or molecules, e.g., cell surface antigens. These may be responsive to UV, visible and IR light (modulated or un-modulated).
2. Photomodulation as a light-generated mediator signal, e.g., production of heat, hypoxia, reactive oxygen species (ROS), and other gas molecules.

Low-level light therapy (LLLT) is an area of intense research at present with a particular focus on using LLLT as a treatment for acne vulgaris, facial dyschromia and rhytids, androgenic alopecia, and wound healing[33]. Nevertheless, further clinical trials are needed to fully understand the safety and efficacy of this technology. There has been equally promising work in the use of photobiomodulation to treat oral mucositis in patients undergoing treatment for cancer[34,35]. There are further recent reports of the benefits of photobiomodulation in wound care but with the cautionary note that clinical protocols need to be optimized by the use of rigorous clinical research studies[36]. Our own work in the field of photobiomodulation has shown that a modulated low-power red laser can activate pluripotent human Very Small Embryonic Like (hVSEL) stem cells in autologous Platelet Rich Plasma (PRP) as discussed below. We proposed a mechanism of action being interactions of modulated laser light with the hVSEL stem cells at the quantum level[37]. We are just at the beginning of understanding and applying photomodulation in regenerative medicine but the future is very bright.

Bioactive Agents in Regenerative Medicine

There are many bioactive agents which may be complementary to regenerative medicine. PRP has for example been important and clinically useful not only in aesthetic (cosmetic) procedures but also in regenerative medicine[38]. Some workers claim that PRP is the 'elixir of youth' when applied for skin rejuvenation and restoration of hair growth[39]. This may be wishful thinking on the part of the authors, but there is no doubt that PRP is a potent tool which has greatly enhanced regenerative medicine. PRP contains high numbers of platelets which produce various cytokines and activation factors, a high concentration of growth factors and cytokines in the plasma itself, and high numbers of pluripotent hVSEL stem cells which will be discussed further below[40].

Another bioactive agent which shows great promise to be complementary to regenerative medicine is Hyaluronic Acid (HA)[41]. HA has been used in various clinical studies, especially in relation to orthopedic disease such as tendinopathies and osteochondral lesions. The combination of HA with stem cell technology may enhance the overall efficacy of such treatments. HA seems to be very effective in orthopaedic disease when given by the intra-articular route[42] and the associated stem cells (e.g., MSC) could be delivered by the same route[43].

Various types of collagens, (sometimes known as the collagen superfamily), have important structural and physiological roles in regenerative medicine[44]. Collagen can be used to create 3D structures which are exceptionally useful in such procedures as tissue grafts. The collagen enables viable stem cell populations to be maintained in grafted tissue, especially in diseases such as corneal scarring[45]. All of these components, and no doubt many more in the future, will have a significant positive benefit on the overall efficacy of regenerative medicine.

Exosomes in Regenerative Medicine

Mammalian exosomes are small acellular vesicles (30-140 nm in diameter) which are released by most mammalian cells. Exosomes are thought to be involved in cell-to-cell communication and cell-to-cell regulation[46]. The current obstacles to bringing exosomes into routine clinical practice include a lack of understanding between exosome structure and function and early-stage development of scalable production[47]. In order to be routinely useful in regenerative medicine then exosomes need to be produced under Good Manufacturing Practice (GMP) conditions in order to ensure safe, consistent, and effective batches of exosomes for clinical use[48]. Despite these hindrances to the overall development of exosome technology, there are reports of clinical-grade exosome production which have been used to treat pancreatic cancer[49], in general cancer therapeutic procedures[50], in wound healing[51] and for drug delivery systems[52]. Our own work using stem cells and exosomes to treat cerebrovascular accidents (CVA) is extremely promising (publication in preparation). It is clear that exosomes have an important role in future therapeutic procedures, and it is inevitable that they will become an important component of regenerative medicine in the future.

Plant-Derived Exosomes (PDE)

Plant-derived exosomes (PDE) are a type of extracellular vesicle and are from 50-100 nm in diameter[53]. These PDE have been shown to contain lipids, micro RNAs, proteins and many other metabolites and it has been observed that some PDE have anti-inflammatory properties[54]. In comparison to mammalian derived exosomes PDE appear to have a higher bioavailability, less immunogenicity and increased innocuity[55].

The key advantages of PDE are:

1. They are biomimetic to mammalian derived exosomes making them an easy, reliable reproducible and safe alternative to mammalian derived exosomes.
2. They are lipophilic and will therefore pass through the skin to the lower dermis and the dermal capillary bed very easily. This means that they can be used as a cosmeceutical thus avoiding complex and expensive licensing procedures.

Plant derived exosomes are certainly an evolving technology in regenerative medicine especially in the treatment of inflammation, chronic pain, burns, anti-aging and the regrowth of hair where they have already shown great potential.

Extracorporeal Shock Wave Therapy (ESWT)

ESWT is a non-invasive therapy which has developed from Extracorporeal Shock Wave Lithotripsy (ESWL)[56]. ESWT is proving increasingly important and effective especially in the field of orthopaedics[57] and sports medicine[58]. The physics of ESWT is relatively complex and beyond the scope if this review, nevertheless there are some excellent reviews on the subject[59]. ESWT relies on a shock or pressure wave transforming into a biological response, this is the so called 'mechnano-transduction'[60-62]. The mechanism of action of ESWT is currently being understood at the molecular level which involves cell surface antigen activation. These activated cell surface antigens are in turn stimulated by the appropriate cytokines and growth factors resulting in the beneficial clinical effects seen[58,59]. A much more detailed understanding will be needed in the future, perhaps even at the quantum level, but at present this work has yet to be completed. It is possible that ESWT activates the Wnt5a/Ca^{2+} signalling in bone marrow mesenchymal stem cells (MSC) which could in turn promote repair and regeneration by these stem cells especially in musculoskeletal disease[63]. Our own experience with ESWT in RegmedGeorgia has so far been extremely encouraging suggesting that ESWT in another technique which has a great deal to offer in the overall application of regenerative medicine.

Platelet Rich Plasma (PRP) in Regenerative Medicine

Platelet-rich plasma (PRP) has been shown to be a valuable therapeutic in regenerative medicine related procedures including the treatment of musculoskeletal disease[64,65] and in the treatment of sports-related injuries[66,67]. PRP was traditionally considered to be a blood product which had a platelet count higher than that found in peripheral blood, making it a possible treatment for thrombocytopenia[68].

Platelets are acellular and they contain four types of granules:

- Alpha granules[69] containing the adhesive proteins fibrinogen, vitronectin, thrombospondin, and von Willebrand Factor (VWF). In addition, alpha granules contain growth factors and cytokines which may mediate wound repair, inflammation, and angiogenesis[70].
- Dense (or delta) granules containing ADP, ATP, calcium, serotonin, polyphosphate, and pyrophosphate[71].
- Lysosomes containing hexosaminidase, arylsulphatase or arylsulfatase, ß-glucuronidase, ß-galactosidase, acid phosphatase, and cathepsins[72].
- T (or tubular) granules containing TLR9, PDI, and VAMP-8 which are thought to be an alpha granule subtype[73]

The platelets in PRP clearly have a potential role in the regenerative process[74]. The plasma in PRP contains high concentrations of growth factors and cytokines including interleukins, RANTES, PDGF, VEGF, GM-CSF, MIP 1b, and CXCL chemokine (IP-10)[75]. These cytokines and growth factors, in concentrated form in PRP, enable differentiation, proliferation, tissue morphogenesis, and chemotaxis in tissue healing[76]. The mechanism of action of these cytokines and growth factors in PRP is proposed to be by the autocrine and paracrine route[77]. PRP is clearly of present and future importance in regenerative medicine. It will, no doubt, be used alone and in combination with cell therapies in the future.

Human Very Small Embryonic-Like (hVSEL) Stem Cells in Regenerative Medicine

The third component of PRP, which is often ignored or dismissed as being present in PRP, is hVSEL stem cells. Research has clearly shown the presence of CXCR4+, SSEA4+, Oct 3/4+, CD45-, Lin- hVSEL pluripotent stem cells in PRP derived from human peripheral blood[78]. The biological and therapeutic action of PRP does not currently include discussions on the importance of hVSEL stem cells in PRP in the overall efficacy of PRP treatments.

The persistence of hVSEL stem cells throughout life has been reported[79] suggesting a potential homeostatic mechanism which maintains the hVSEL stem cell pool throughout life. The bone marrow is the likely source of the hVSEL stem cells. This is supported by the observation of mobilization of hVSEL stem cells into the peripheral blood following acute myocardial infarction[80]. In addition, hVSEL stem cells have been shown to be present in the human bone marrow and also in human leukapheresis products[81]. This supports the hypothesis of migration of hVSEL stem cells from the bone marrow to the peripheral blood during physiological homeostasis and during pathological stimuli. Similar studies *in vitro* have suggested that hVSEL stem cells are the 'original embryonic stem cell' highlighting the critical importance of hVSEL stem cells in normal embryonic development and subsequent physiological homeostasis[82].

All stem cell types are subject to both intrinsic and extrinsic stress during normal physiology and in pathological states. Such stress can have detrimental effects, especially on rapidly dividing stem cells. There is a population of quiescent VSEL stem cells in murine bone marrow which may be resistant to extrinsic heat stress in the same way as the quiescent population of MSC derived from desquamated endometrium of menstrual blood (eMSC)[83]. This raises the possibility that quiescent hVSEL stem cells residing in the bone marrow may be available for collection and QiLaser activation following high-dose chemotherapy. If this can be proven, then quiescent hVSEL stem cells may offer an alternative therapeutic route to patients who have undergone chemotherapy with the resultant damage to normal somatic cells. Such an approach may have the ability to enhance somatic cell and tissue repair following high dose chemotherapy[84].

QiLaser Activation of PRP

Standard autologous PRP treatments usually involve the collection of peripheral blood into citrate dextrose anticoagulant, centrifugation at room temperature, and then simple reinfusion of the room temperature PRP back into the patient. Such preparation of inactivated PRP may result in premature platelet activation which can be modulated by introducing thrombin into the PRP[82]. Research has clearly shown that QiLaser (previously known as the SONG modulated laser) modulated laser light interacts with hVSEL stem cells in PRP to upregulate the expression of CXCR4, Oct 3/4, and SSEA4[78]. The use of light as a method of activation stem cells has also been confirmed by other workers[86]. The proposed mode of action for QiLaser modulated laser light on hVSEL stem cell activation

has been made using concepts taken from quantum physics[87]. The QiLaser can also be applied to the patient as part of the treatment, to the areas where the stem cell repair is needed e.g. to the head in the case of neurodegenerative disease. This is supported by other authors who propose that enhanced homing of stem cells may improve the efficacy of regenerative medicine procedures[88]. The benefits of QiLaser activated hVSEL stem cells are that they are autologous, they are very easily collected from the peripheral blood and PRP prepared, and they can be used to treat a wide range of diseases. This is because hVSEL stem cells are pluripotent. There is currently no other readily available source of pluripotent human stem cells for clinical use.

Conclusions

Regenerative medicine is developing at a rapid rate. Cellular-based therapies are slowly being introduced and acellular therapies, such as those described above, have entered routine clinical use. RegMedGeorgia will lead the way in the use of innovative regenerative medicine technologies along with colleagues around the World.

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Professor Peter Hollands is a Freelance Consultant Clinical Scientist. Dr Tengiz Tkebuchava is Medical Director and CEO of RegMedGeorgia

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